ESTIMATION OF CIRCULATING IMMUNE COMPLEXES

IN PATIENTS WITH ORAL LEUKOPLAKIA AND

ORAL SUBMUCOUS FIBROSIS – A CASE CONTROL STUDY.

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CERTIFICATE

This is to certify that this dissertation titled **"Estimation of Circulating Immune Complexes in patients with Oral Leukoplakia and Oral submucous fibrosis – A Case Control Study"** is a bonafide record of work done by **"Dr.H.MAHESWARI"** under my guidance during her post-graduate study period between 2008 – 2011.

This dissertation is submitted to **THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY** in partial fulfillment for the **Degree of Master of Dental Surgery in Branch IX – Oral Medicine and Radiology.**

It has not been submitted (partial or full) for the award of any other Degree or Diploma.

Guided By:

20/9/10

Dr.Capt.S.Elangovan. M.D.S.,

Professor,
Dept. of Oral Medicine & Radiology,
Ragas Dental College & Hospital,
Chennai – 600 119.

Dr. (CAPT) S. ELANGOVAN, M.D.S.,
Prof. Oral Medicine & Radiology
RAGAS DENTAL COLLEGE & HOSPITAL
2/102, East Coast Road Uthandi,
Chennai-600 119.

20/9/10

Dr.S.Shanmugam. M.D.S.,

Professor and Head,
Dept. of Oral Medicine and Radiology,
Ragas Dental College & Hospital,
Chennai – 600 119.

Dr. S. SHANMUGAM, M.D.S.,
Prof. & H.O.D. Oral Medicine & Radiology
RAGAS DENTAL COLLEGE & HOSPITAL
2/102, East Coast Road Uthandi
Chennai - 600 119.

Dr.S.Ramachandran. M.D.S.,

Principal,
Ragas Dental College & Hospital
Chennai – 600 119.

PRINCIPAL
RAGAS DENTAL COLLEGE & HOSPITAL
CHENNAI-600 119.

Place: Chennai – 119.

Date: 20.09.2010.

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SL.NO	ABBREVIATION	EXPANSION
1.	CIC	Circulating Immune Complex
2.	ELISA	Enzyme Linked Immuno Sorbent Assay
3.	Ig	Immunoglobulin
4.	LAI	Latex Agglutination Immunofluorescence
5.	OL	Oral Leukoplakia
6.	OSCC	Oral Squamous Cell Carcinoma
7.	OSMF	Oral Sub Mucous Fibrosis
8.	PEG	Poly Ethylene Glycol
9.	WHO	World Health Organization

Hippocrates ,the father of Medicine (460 B.C.-375 B.C) coined the term "Karkinos" for the non healing neoplastic ulcers and "Karkinoma'' for the solid malignant tumours. Both the terms derived from Greek. "Karkinos" means "Crab''. The reason behind the terminology is the malignant tumors adhere to any part ,which it seizes upon in an obstinate manner like crab.[18]

The term "Oral cancer'' is used to describe any malignancy that arises from the oral tissues. Oral cancer is the most life threatening disease of oral tissues, causing a major health problem in India. [18]

Oral cancer is the sixth most common cancer worldwide and continues to be the most prevalent cancer related to the consumption of tobacco, arecanut, alcohol and other carcinogenic products. Increasing awareness on part of the providing treatment, as well as the population in general, has led to a large proportion of patients presenting with earlier stage of the disease.[31]

Several studies have shown clearly that oral cancers either develop from precancer or are associated with it. The problem of oral precancer and cancer in India has been investigated extensively and its incidence has been shown to be really high.

Epidemiological studies indicate that intervention at an early stage might reduce oral carcinoma related morbidity and mortality. This includes detection of oral cancer at its precancerous stage. The discovery of immunological markers at blood level, histological and molecular level has revolutionized the diagnosis and prognosis in oral precancerous and cancer lesions.[15]

Oral cancer is generally preceded by some precancerous lesions and conditions for a varying length of time. They share the same etiological factors with oral cancer, particularly the use of tobacco and areca nut exhibit the same site and habit relationship.

Precancerous lesion is a morphologically altered tissue in which cancer is more likely to occur than its apparently normal counterpart. Leukoplakia and Erythroplakia are precancerous lesions. Precancerous condition is defined as generalized state associated with a significantly increased risk for cancer. Oral submucous fibrosis and Erosive lichen planus are precancerous conditions .[12]

Individuals with oral precancer such as oral leukoplakia and oral submucous fibrosis run a risk that is 69 times higher for them to develop oral cancer compared to tobacco users who do not have pre cancer. The recognition and management of precancerous lesions and conditions therefore constitutes a vital oral cancer control measure.[49]

In India, oral cancer is prevalent in most areas where tobacco related practices are observed. For development of oral cancer, tobacco is the single greatest risk factor. This is due to higher concentration of carcinogenic exposure and failure to clean the carcinogens from the mucosal surface.

Alcohol, areca nut, viruses, sunray, smoking, genetic mutations, Syphilis,chronic irritation and diet deficiency states are also implicated in the etiology. The development of oral cancer is a multistep process arising from pre-existing potentially malignant lesions. Leukoplakia is the most common precancer representing 85% of such lesions.

Likewise, the incidence of Oral Submucous Fibrosis (OSMF) is increasing like an epidemic, targeting the younger generation. The etiology for OSMF is still obscure and a varied number of factors have been proposed. Of these, areca nut use is the most important and persistent finding in history taking.[1,49]

Immune complexes are clusters of interlocking antigens and antibodies. Under normal conditions immune complexes are rapidly removed from the bloodstream by macrophages in the spleen and Kupffer cells in the liver. In some circumstances, however, immune complexes continue to circulate.[6]

Immune complexes may themselves cause disease when they are deposited in organs,such as in certain forms of vasculitis. This is the third form of hypersensitivity in the Gell-Coombs classification, called Type III hypersensitivity.[33]

Immune complex deposition is a prominent feature of several autoimmune diseases, including systemic lupus erythematosus, cryoglobulinemia, rheumatoid arthritis, scleroderma and Sjögren's syndrome.

Circulating immune complex disease occurs when the host's antibody production, relative to the amount of antigens, is inadequate for prompt elimination of antigen. Normally, excess amounts of antibody are formed which generate large immune complexes that are removed very rapidly from the circulation and are disposed of by the mononuclear phagocytic system. If the antibody response is very poor, only a few very small complexes are formed and are prone for vascular deposition.[33]

When the relative antibody production is such that complexes of intermediate size form, vascular trapping can occur and injury results from the effects of inflammation. In addition to immune complex size, other factors influence vessel deposition, including the efficiency of systemic clearance of immune complexes, the hemodynamics of blood flow, and vasoactive amine-influenced changes in vascular permeability.[33]

Intensive studies have documented the role of immune complexes as modulators of both cellular and humoral immune response. The occurrence of Circulating Immune Complexes (CIC) as a marker for tumor burden and prognosis in the sera of patients with oral precancer and cancer is now well established.[64,65]

Recent advances in the fields of Circulating Immune Complexes, tumor progression, drug resistance, tumor cell heterogeneity and metastasis have resulted in a renewed interest in the development of non- specific immunotherapeutic modalities .

The overall consensus is that only a small percentage of the detected CIC in vivo represent tumor associated antigens complexed with antibodies. The bulk of CIC most likely represent auto antibodies or the reaction to denatured self proteins, microbes, normal lymphocyte, antigens and nuclear antigens. Antigenic make up of CIC in cancer patients reflects the host's immune response to a variety of often overlapping antigenic stimuli and hence paves way for further studies.[43]

Immunological and biochemical alterations in the sera of such patients can help not only in early diagnosis, appropriate treatment but also as indicators of prognosis, as the disease progresses. CIC represent the host's physiological and immunological defense response in eliciting specific antibodies upon exposure to most antigenic substances..[54]

This study was conducted to understand the role of these CICs in the oral potentially malignant disorders like oral leukoplakia and OSMF. It can be suggested that immunological assessment of markers such as CIC in oral precancer patients may help in earlier diagnosis and prognosis of these lesions/conditions. Since ,CIC levels in Serum helps in predicting malignant potential of the pre malignant lesions/conditions it can be used as a reliable biomarkers.

Aim:

- To validate Circulating immune complexes as biological marker for Oral Leukoplakia and Oral submucous fibrosis.

Objectives:

1. To determine the levels of Circulating immune complexes in subjects with untreated Oral Leukoplakia and untreated Oral submucous fibrosis.

2. To compare the Circulating immune complexes in Oral Leukoplakia and Oral submucous fibrosis levels with the control group.

3. To detect the relation between circulating immune complexes with oral leukoplakia and Oral submucous fibrosis.

The study is about the Estimation of Circulating immune complexes in Oral leukoplakia and Oral sub mucous fibrosis patients in comparison with healthy normal controls. To obtain a meaningful study and result, a proper and detailed review of the literature is of utmost importance. The present literature review is about the various aspects of Oral leukoplakia, Oral sub mucous fibrosis, and their correlation with Circulating immune complexes levels.

PRECANCEROUS LESIONS AND CONDITIONS:

Precancerous lesion:

Precancerous lesion is a morphologically altered tissue in which cancer **is** more likely to occur than its apparently normal counterpart.

It includes: Leukoplakia, Erythroplakia and palatal lesions in reverse smokers.[101]

Precancerous condition:

Precancerous condition is generalized state associated with significantly increased risk of cancer.

It includes: Oral sub mucous fibrosis, Actinic keratosis, Lichen planus and Discoid lupus erythematosis, Syphilis and Siderophenic dysphagia.

A new terminology has been recommended as "Potentially malignant disorders" which included not only the lesions and conditions but also includes a family of morphological alterations among which few may

have an increased potential for malignant transformation. These potentially malignant disorders have been further classified as lesions and conditions. But currently they recommend not dividing them as such, but instead clubbing them together under the heading of "Potentially malignant Disorders". [100,101]

ORAL LEUKOPLAKIA:

Historical review:

Erno Schwimmer in 1877 [37,] a Hungarian dermatologist was the first person to describe Oral Leukoplakia. He originally proposed the term "Leukoplakia" which literally means "White patch". In Greek "Leukos" means white and "Plakia" means patch. It describes a clinical entity.

Sprage in 1963 [37] found that 36% of American oral pathologists used Oral leukoplakia as clinical term and 40% as histological one.

Shafer in 1967 [37] suggested that histological definition of leukoplakia is however complicated by the fact that not all lesion satisfying the histological criteria for leukoplakia will appear clinically as a white patch.

W.H.O Collaborating Centre for Oral Precancerous Lesions in 1978 [37] proposed that the term Leukoplakia should be used to describe a clinical entity, that is a white patch of oral mucosa which is not removed by rubbing and not classifiable as any other oral disease like oral lichen planus, leukedema, candidiasis ,white sponge nevus, lupus erythematosus . It also

suggests that microscopically leukoplakic lesions should be described in internationally approved term.

Brad W. Neville et al in 2002 [9] proposed that as such leukoplakia should be used only as a clinical term and it has no specific histopathological connation should never be used as microscopic diagnosis. Sometimes a white patch is initially believed to represent leukoplakia, but the biopsy reveals another specific diagnosis. In such cases, the lesion should no longer be categorized as leukoplakia.

Definition of leukoplakia:

WHO collaborating centre for oral precancerous lesions in 1978 [37] defined oral leukoplakia as "A white patch or plaque that cannot be characterized clinically or pathologically as any other disease."

Axell T et al in 1996 [2] defined leukoplakia as "A white patch or plaque that cannot be characterized clinically or pathologically as any other disease and is not associated with any physical or chemical causative agent except the use of tobacco".

Axell T et al in 1996 [2] also defined oral leukoplakia as "A predominantly white lesion of oral mucosa that cannot be characterized as any other definable lesion; some leukoplakia will transform in to cancer."

Pindborg et al in 1997 [58] defined leukoplakia as "A predominantly white lesion of the oral mucosa that cannot be characterized as any other definable lesion".

WHO in 2005 [101] declared "Leukoplakia should be used to recognize white patch of questionable risk having excluded other known diseases or disorders that carry no increased risk for cancer"

Epidemiology:

In a 10 year prospective study in India in large samples, carried out in several geographic areas with various kinds of tobacco usage, the annual age- adjusted incidence rates of leukoplakia per 1000 population per year varied from 1.1 -2.4 among men and from 0.2 -1.3 among women and the prevalence varied from 0.2-4.9%. [29]

The annual incidence was 2.1 per 1000 men 1.3 peer 1000 women and the highest being in people with mixed habits, that 6 per 1000.[99]

Prevalence of leukoplakia in India varies from 0.2-5.2%. Prevalence of 0.59 also has been reported. [80]

Prevalence of leukoplakia was reported to be 3.6% and that of preleukoplakia was 6.4%. Idiopathic leukoplakia was reported to be 0.7% and tobacco specific leukoplakia was 2.9%. [2]

In Ernakulam district the prevalence of leukoplakia was 17 per 1000 population. It was more commonly seen in people with mixed habit.

Less than 15 of men below the age of 30 years, have leukoplakia, but the prevalence increases to an alarming 8% in men over the age of 70 years. The prevalence in women past the age 70 years is approximately 2%.[8]

Age:

The onset of lesions usually starts after 30 years, resulting in peak incidence of 50 years. Leukoplakia is seen most frequently in middle aged and older men, with an increasing prevalence with age. Oral leukoplakia can occur 5 years prior to oral cancer.[36]

Gender:

It has a strong male preponderance. Leukoplakia is a commonly occurring lesion particularly in patients after 40 years of age. The male to female ratio is 2:1. The gender distribution in most studies varies, ranging from a strong male predominance in different parts of India, to almost 1:1 in Western world.[96]

Etiology:

Many causative agents have been implicated in the etiology of leukoplakia. They include Tobacco, Alcohol, Chronic friction due to sharp tooth, Electro galvanic reaction, Ultra violet radiation, Syphilis. [36.]

Tobacco use is considered to be the primary cause for the occurrence of leukoplakia. Leukoplakia associated with smoking habit seems to have less malignant potential than those not related to smoking habit. In a study of 257 patients with oral leukoplakia, 183 were smokers, out of whom 12% developed carcinomas, whereas 74 were non- smokers out of whom 32% developed carcinoma. [86]

Local factors like chronic irritation and malocclusion which constantly irritate the oral mucosa cause oral leukoplakia. Irritation by ill

fitting dentures, overhanging crowns and fillings and poor restoration refers to traumatic causes. Smoking is also considered as an irritant due to drying effect on the mucosa. These exciting factors should act on a susceptible host. Heredity plays an important role in determining an individual's susceptibility or resistance to the development of leukoplakia. [85]

Systemic factors include hormonal alterations and nutritional deficiencies. Estrogen deficiency may determine the susceptibility of females to develop leukoplakia at the period of menopause.

Vitamin A deficiency may produce Hyperkeratosis in experimental animal. Continuous irritants such as spicy food may also be a factor. Irritating mouthwashes can also be a cause but the exact role is yet to be substantiated.

Syphilis may also be responsible for leukoplakic lesions on tongue secondary to atrophic glossitis of tertiary syphilis. The glossitis is apparently more highly susceptible to the action of local irritants in the mouth. [36]

In one preliminary study Epstein Barr virus was detected in 60% of Proliferative verrucous leukoplakia cases suggesting its possibility as an etiological agent.

Yeast organism especially candida albicans can cause leukoplakia since it has the ability to form N-Nitrosomethylamines from the precursors which are abundantly available in the saliva of smokers.

To list them under a table as [36]:

Local	Systemic
Local irritation • Sharp ,malposed teeth • Ill fitting denture • Poor restorations	Heredity
Occlusal disharmony	Harmonal factors
Occlusal habit	Estrogen deficiency
Thermal factors	Nutritional deficiency
Smoking	Syphilis
Irritant foods,chemicals,mouthwashes, etc.	Atrophic glossitis

Clinical types [7, 100] :

Two main types exist:

- Homogeneous
- Non homogeneous

Distinction between these two forms is purely clinical, based on surface color and morphological characteristics like thickness which also has predilection for prognosis.

Homogeneous type:

Homogeneous leukoplakia has been defined as a predominantly white lesion of uniform flat, thin appearance that may exhibit shallow cracks

and has a smooth, wrinkled or corrugated surface with a constant texture throughout. The risk of malignant transformation is relatively low. The lesion is predominantly white but can be grayish white. It constitutes for about 84% of the leukoplakia.

Non homogeneous type:

- Ulcerative: Mixed white and red in color but retaining the predominant white character.
- Nodular (Speckled): Small polypoid outgrowths, rounded red or white excrescences.
- Verrucous: wrinkled or corrugated surface appearance.

The term "Erythro leukoplakia" is applied for predominantly red and white lesion that may be irregularly flat, nodular or exophytic. The nodular lesions are characterized by white patches or nodules on a erythematous base.

Ulcerative leukoplakia:

Ulcerative leukoplakia is characterized by areas of red which at times exhibit yellowish areas of fibrin. White patches are generally present at the periphery. It constitutes to about 135 of all leukoplakia types. It could also present with ulceration with white areas in between. Some times they occur with minimal keratinisation.

Speckled leukoplakia:

Speckled leukoplakia is also called Nodular leukoplakia. It is characterized by small white specks or nodules on an erythematous base. The term nodular leukoplakia was proposed by Pindborg et al in 1968 [57] based on the findings at the labial commisure. The nodules are very fine, speckled, pin head sized or even larger. It constitutes to about 3% of leukoplakia. It is important lesion because of its high risk for malignant transformation

Verrucous leukoplakia;

Verrucous leukoplakia appears as an exophytic lesion with irregular sharp or blunt projections.

Clinical features:

Oral leukoplakia accounts for 80% of all leukoplakia of upper aero digestive tract. Most of the others are vocal cord lesions and are called as laryngeal keratosis. It can occur any where in the oral cavity. The site of leukoplakia depends on the type of smoking habit, the quality and the quantity of the tobacco.

Most commonly involved sites are retro commissural area, buccal mucosa, edentulous alveolar ridge, hard palate, tongue, lips. The gingival, soft palate and floor of mouth are less commonly involved in an Indian population, where as it is not true for Western population.

Leukoplakia begins as thin, gray white plaques that may appear somewhat translucent, sometimes fissured or wrinkled and are soft and flat.

They usually have sharply demarcated borders but occasionally blend gradually in to normal mucosa. This early stage is considered as Phase –I Leukoplakia.

Thin leukoplakia may continue unchanged or disappear, but with time 2/3rd of these lesions acquire a distinct white appearance from a thickened keratin layer. It becomes leathery to palpation and fissures deepen. There may be few or localized nodules or appear as surface projections. This can be categorized as Phase-II.

Most of these lesions remain unchanged and about 2/3rd regress and disappear and few become severe. Later these lesions develop surface irregularities of a nodular or granular leukoplakia. Numerous pointed projections develop on the surface and give an appearance of verruciform leukoplakia. Both these lesions are Phase-III lesions with similar progress. Phase-III lesions may become dysplastic or invasive with no change in clinical appearance.

Over time an additional surface change occurs and multiple circular or oval patches of non blanching redness appear in scattered areas. Such areas represent site at which cells are so immature that they are unable to produce keratin. These forms Phase-IV lesions.

Leukoplakia Clinical phases [36]:

Phase	Descriptive terms	Risk of malignant transformation
I	Thin leukoplakia Preleukoplakia Homogeneous leukoplakia	+/-
II	Thick,smooth leukoplakia Fissured leukoplakia Homogeneous leukoplkia	++
III	Granular leukoplakia Verruciform leukoplakia Rough leukoplakia Candidal epithelial hyperplasia Homogeneous leukoplakia	+++
IV	Erythroleukoplakia Speckled leukoplakia Candidal leukoplakia Nonhomogeneous leukoplakia	++++

Pindborg et al in 1997 [58] has given the classification and staging for leukoplakia as follows,

Classification and staging of oral leukoplakia

Provisional (Clinical Diagnosis)

L: Extent of leukoplakia

L0: No evidence of lesion

L1: ≤ 2 cm

L2: 2-4 cm

L3: ≥ 4cm

S: Site of leukoplakia

S1: all sites excluding floor of mouth & tongue

S2: floor of mouth &/ tongue

S3: not specified

C: Clinical aspect

C1: homogeneous

C2: non homogeneous

C3: not specified

Definitive diagnosis :(Histopathological diagnosis)

P: Histopathological features

P1: no dysplasia

P2: Mild dysplasia

P3: Moderate dysplasia

P4: severe dysplasia

Px: not specified

Staging:

1. any L,S1,C1,P1 or P2

2. any L,S1or S2,C2,P1 or P2

3. any L,S2,C2,P1 or P2

4. any L,any S,any C,P3 or P4.

Natural history:

Leukoplakia can regress spontaneously without any intervention in habit or by any other means in about 40% of cases. Significantly higher rates of regression is seen who discontinue the tobacco habit. In one long term follow-up study among the Swedish population consisting 104 samples, they found that oral leukoplakia has disappeared in 43% of the people. About 70-80% of leukoplakia is associated with tobacco habits; also about 80% of the leukoplakia lesions disappear completely about 58% or regress within 12 months after smoking cessation.[11]

Malignant transformation:

It is generally accepted that dysplastic lesions carry a 5 fold greater risk than non dysplastic ones. It refers to the development of oral cancer from preexisting oral leukoplakia. So it is necessary to follow-up a case of leukoplakia for a period of 3 months to one year. [12]

In the period of follow-up, the lesion should be evaluated for development of thickened/nodular areas, ulcerations, rolled margins, growths or indurated areas. Since these changes represent early oral cancers. Lesions on the tongue, lip vermilion border, floor of the mouth accounts for 93% of the leukoplakia with dysplastic changes or carcinoma. Globally 3-6% leukoplakia change to cancer.[27, 28, 84]

Non homogeneous leukoplakia accounted for the highest frequency of malignant transformation of 20%, whereas 3% of the homogeneous leukoplakia developed carcinoma. Proliferative verrucous leukoplakia has a malignant transformation rate as high as 70.3% with mean follow-up of 11.6% [12, 25, 51]

Differential diagnosis [44, 55]:

The following differential diagnosis should be kept in mind whenever a clinical diagnosis of leukoplakia is made:

- Surface debris. This can be scrapped off with a tongue blade or gauze.
- Acute pseudo membranous *candidiasis* (Thrush). White, curd-like or cotton like patches or plaques, most frequently occurring on the buccal mucosa and tongue, but also seen on the palate, floor of mouth and gingiva. They are usually associated with a burning sensation and the white plaque can usually be wiped away with gauze, leaving a tender, red area beneath, which may bleed.

- Reactive hyperkeratosis. A benign epithelial response, usually due to trauma from a fractured tooth or dental restoration. Check for the causative agent.
- Leukedema :Usually bilateral, that disappears on stretching the mucosa.
- Lichen planus : Usually bilateral with lacy white pattern.
- Lichenoid reaction: same like lichen planus;but positive history of drug intake, amalgam restoration, pan chewing habit.
- Linea Alba buccalis : Usually bilateral. The white line is present along the occlusal plane.

Diagnosis :

Axell in 1996 [2] suggested that diagnosis of leukoplakia can be arrived at by excluding lesions belonging to other entities, such as Lichen planus,Lupus erythematosus, Leukedema and White sponge nevus and lesions for which an etiology can be established ,such as frictional keratosis, cheek/lip/tongue biting, contact lesions and smokers palate.

Pindborg in 1997 [58] proposed that when there is a doubt in diagnosing leukoplakia ,one can make a provisional diagnosis of leukoplakia and the definitive diagnosis can be established after the result of removal of any possible etiologic factors and a biopsy.

Review on oral leukoplakia:

Racugno V, Cossu F in 1969 [63] , reviewed influence of tobacco smoke on the origin of leukoplakia and carcinomas of the mouth, with

special reference to smokers of cigars with the lighted end in the mouth and found there is significant relation between tobacco smoke and leukoplakia.

Sugár.L. et al in 1969 [90], made a follow-up study with 324 patients and concluded that there was a relationship between smoking and the frequency of leukoplakia. He also found that alcohol, mechanical irritants and electric potential difference in the mouth were also important etiological factors, particularly in combination. Many of the cases with mild symptoms improved when the etiological agent was removed.

Waldron C A and Shafer W G. in 1975 [99] made a clinico pathologic study with 3256 oral leukoplakia cases . During a 13-year period, 3256 specimens clinically diagnosed as leukoplakia (('keratosis," "white patch") were analyzed as to age of occurrence, site of involvement.

It was found that: leukoplakia occurs chiefly in the 5th, 6th, and 7th decades; about half of the lesions involved the mandibular mucosa, mandibular sulcus, and buccal mucosa; leukoplakia was slightly more common in men (54.2%).

Bánóczy J in 1977 [5] made a follow-up study with 670 patients with oral leukoplakia during a 30-year-period showed cancer development in 40 cases, The age distribution revealed the prevalence of leukoplakia in the age-group 51-60 years; that of carcinoma in the age-group of 61-70 years. The sex distribution showed a male-female ratio of 3.2: 1 in the leukoplakia-group, and a 1.9: 1 ratio in the carcinoma-group. The tongue and the lips were the site of predilection for malignant transformation and for dysplasia.

Among etiological factors, Candida albicans infection and the simultaneous existence of several etiological factors seemed to play a role in malignant transformation. Erosive leukoplakia showed the highest risk, developing in 25.9% of the cases into cancer.

Sciubba JJ. in 2001 [81] reviewed the importance of early diagnosis and treatment of oral cancer and suggested that there is strong evidence for an etiological relationship between human papilloma virus and a subset of head and neck cancers. It is generally accepted that most sporadic tumors are the result of a multi-step process of accumulated genetic alterations. These alterations affect epithelial cell behavior by way of loss of chromosomal heterozygosity which in turn leads to a series of events progressing to the ultimate stage of invasive squamous cell carcinoma.

The corresponding genetic alterations are reflected in clinical and microscopic pathology from hyperplasia through invasiveness. A wide range of mucosal alterations are closely related to leukoplakia. Proliferative verrucous leukoplakia represents a relatively new type of leukoplakia that is separate from the more common or less innocuous form of this condition. Erythroplakia is particularly relevant considering its almost certain relationship with dysplasia or invasive carcinoma.

Squamous cell carcinoma will develop from antecedent dysplastic oral mucosal lesions if an early diagnosis has not been made and treatment given. Early diagnosis within stages I and II correspond to a vastly improved 5-year survival rate when compared with more advanced stage III

and IV lesions. Surgical management of this disease remains the mainstay of treatment. Other therapies include radiation and chemotherapy options that may be used adjunctively and palliatively. Following treatment, it is important to understand the significant risks of second primary cancers developing within the upper aero digestive tract as a result of field cancerization. The most important message is that early detection of the asymptomatic early stage oral cancer translates in general terms to satisfactory clinical outcome and cure in most patients.

Dietrich T in 2004 [19] made an analytical study on Clinical risk factors of oral leukoplakia and found the results as, Tobacco smoking as the strongest independent risk factor. The Odds Ratio were 3.00 (0.77-11.8) for < for =10 cigarettes/day and up to 6.01 (2.4-15.0) for >20 cigarettes/day. Diabetes, age and socio-economic status were found as independent predictors of Oral leukoplakia. Alcohol consumption, race/ethnicity, years of education and Body Mass Index showed no independent association with Oral leukoplakia Females with a history of estrogen use were less likely to have Oral leukoplakia with an Odds ratio of 0.34 (0.11-1.07).

Chung CH et al in 2005 [12] made a study to find the relation between Oral precancerous disorders with areca quid chewing, smoking, and alcohol drinking in southern Taiwan and found of 1075 subjects,136 12.7%). Precancerous lesions and conditions were detected .The analysis of the spectrum of oral precancerous disorders detected, leukoplakia (n = 80), OSMF (n = 17) and verrucous lesions (n = 9), demonstrated an association

with gender ($P < 0.001$). There were statistically significant associations among leukoplakia ($P < 0.01$), OSMF ($P < 0.0001$), and verrucous lesions

The synergistic effect of smoking and areca quid chewing habit on leukoplakia and OSMF was demonstrated. Conclusion of this study reinforced the association of current areca quid chewing without tobacco, cigarette smoking, and alcohol drinking to leukoplakia, Oral submucous fibrosis, and verrucous lesions in Taiwan.

Lee JJ et al in 2006 [40] conducted a study to find the relation between carcinoma and oral leukoplakia and demonstrated the results that some leukoplakias contain a malignant component. Lesions with certain features are more prone to carcinoma, but no clinical attributes can bring certainty in these lesions.

Prakash C.Guptha in 2006 [60] made an Epidemiologic study of the association between alcohol habits and oral leukoplakia. The study included 10914 individuals for their tobacco and alcohol habits and examined for the presence of oral leukoplakia. Very few females (1.6%) were found to be alcohol users and they were excluded from further analysis. Among 7604 males, 30.4% used alcohol regularly, 25.4% occasionally and 44.2% were non-users. The prevalence of leukoplakia was significantly higher among regular (5.7%) and occasional (3.9%) users than among non-users (2.9%) of alcohol.

Alcohol usage was found to be related to age as well as tobacco habits. The prevalence of leukoplakia was higher among alcohol users in

each age-group as well as in each tobacco habit category. After age-adjustment the difference between alcohol users and non-users, although reduced, remained significant. For most tobacco habit categories the trend remained similar after age-adjustment except for the mixed habits group, for which there was a reversal of the trend. The alcohol habit may, perhaps, produce discernible effects only in association with other 'weak' etiological risk factors, such as a single tobacco habit of smoking or chewing rather than a 'strong' etiologic factor such as the mixed habits of chewing and smoking.

Sol Silverman in 2006 [86] made a follow up study on oral leukoplakia and Malignant transformation in 257 patients for average period of 7.2 years .Seventy-three percent of the patients used tobacco, with cigarette usage being the predominant form. Forty-five patients (17.5%) subsequently developed squamous carcinomas . High risks for malignant transformation also included non-smoking patients, the clinical presence of erythroplasia (erythroleukoplakia), and a clinical verrucous-papillary hyperkeratotic pattern. Duration of the leukoplakia progressively increased the total number of malignant transformations, with the largest rate occurring in the second year. This study confirms that oral leukoplakia is a precancerous lesion and that certain characteristics indicate greater risks and warrant consideration of more aggressive management.

Thomas SJ et al in 2008 [94] conducted a cross sectional study on Betel quid not containing tobacco and oral leukoplakia .The study recruited

1,670 adults. They recorded betel quid chewing and smoking. The prevalence of leukoplakia was 11.7%. In the nested case-control study of 197 cases and 1,282 controls, current betel chewing was associated with increased risk of leukoplakia with an adjusted odds ratio for current chewers of 3.8 (95% CI 1.7, 8.4) and in the heaviest chewers of 4.1 (95% CI 1.8, 9.1) compared to non-chewers. Current smoking was associated with an increased risk of leukoplakia with an adjusted odds ratio for current smokers of 6.4 (95% CI 4.1, 9.9) and amongst heaviest smokers of 9.8 (95% CI 5.9, 16.4) compared to non-smokers. The systematic review identified 5 studies examining risk of leukoplakia associated with betel quid chewing in populations where betel quid did not contain tobacco and that controlled for smoking. In studies that adjusted for smoking, the combined random effect odds ratio was 7.9 (95% CI 4.3, 14.6) in betel quid chewers. The results of this study and systematic review of similar studies provide evidence of the role of betel quid not containing tobacco and leukoplakia

Van der Waal I in 2009 [97] reviewed Potentially malignant disorders of the oral and oropharyngeal mucosa; terminology, classification and present concepts of management and gave WHO workshop recommendations such as to abandon the distinction between potentially malignant lesions and potentially malignant conditions and to use the term potentially malignant disorders instead. Of these disorders, leukoplakia and erythroplakia are the most common ones. These diagnoses are still defined by exclusion of other known white or red lesions. In spite of tremendous

progress in the field of molecular biology there is yet no single marker that reliably enables to predict malignant transformation in an individual patient. The general advice is to excise or laser excision of any oral oropharyngeal leukoplakia/erythroplakia, if feasible, irrespective of the presence or absence of dysplasia. Nevertheless, it is actually unknown whether such removal truly prevents the possible development of a squamous cell carcinoma

ORAL SUBMUCOUS FIBROSIS :

Historical review :

Oral submucous fibrosis has been well established in Indian medical literature since the time of Sushrutha renowned Indian Physician who lived in the era 2500-3000 B.C.[56]

Schwartz J in 1952, [56] first described Oral submucous fibrosis in modern literature among five East African women of Indian Origin under the term " Atropica idiopathica mucosa oris". Since then this condition has been described by various investigators by various terms.

Joshi S G in 1953,[56] described this condition in India when he reported 41 cases with Oral submucous fibrosis of the palate and it was he who suggested the use of the term "Oral submucous fibrosis''.

Lal.D in 1953 [56] described it as a "Diffuse Oral submucous fibrosis",Joshi S.G in 1953 described it as "Sub mucous fibrosis of the palate and pillars",Sui-Pin in 1954 described it as "Idiopathic scleroderma of the mouth",Rao.A.B.N in 1962 termed it ias "Idiopathic palatal

fibrosis",Behl P.N in 1962 arrtibuted the term "Sclerosing stomatitis''.

Definition:

The most widely accepted definition is by **Pindborg J.J and Sirsat SM in 1966** [56]. They have defined Oral submucous fibrosis as "An insiduous chronic disease affecting any part of oral cavity and sometimes the pharynx, although occasionally preceded by vesicle formation it is always associated with juxta –epithelial inflammatory reaction followed by a fibro elastic change of the lamina propria,with epithelial atrophy leading to stiffness of the oral mucosa and causing trismus and inability to eat."

Schwartz in 1952[56]**,** coined the term atrophica idiopathica mucosa oris to describe an oral fibrosing disease .He discovered in 5 Indian women from Kenya.

Joshi in 1953 [56] subsequently coined the term Oral sub mucous fibrosis (OSMF) for the condition.

Epidemiology:

Oral submucous fibrosis is an epidemic in India, and is prevalent throughout Indian sub continent, sparing no caste or creed, affecting the young and old, the rich and poor alike.[10]

Pindborg J.J et al in 1966 [56] reported that the prevalence of Oral submucous fibrosis in India ranges from 0.2-0.5%wiht a higher predominance in the southern part of the Indian subcontinent.

Pindborg J.J in 1968 [57] conducted an epidemiological survey comparing the incidence of Oral sub mucous fibrosis in Urbanized Indians

with those of rural Indians and observed that the incidence varied from 0% in Bihar to 0.4% in Kerala in rural Indians and 0.18% in Bangalore to 1.22% in Trivandrum in urbanized Indians. They concluded that Oral sub mucous fibrosis is more prevalent in South India than in North India.

Reports from Western India gives an incidence of 2.6 and 8.5 per 1,00,000 per year for males and females respectively. In South India it is 9 and 20 per 1,00,000 per year for males and females respectively.

He stated that South Indian state of Kerala, one new case could be expected per 10,000 populations per year. The prevalence rate of oral submucous fibrosis in South Africa, Burma and India range from 0-1.2%.

Gupta P.C et al in 1989 [29] ,reported the incidence of Oral submucous fibrosis in Ernakulum ,India as 8 per 10,000 per men and 19 per 10,000 women per year. In India Bhavnagar, in Western India the incidence was 2.6 per 10,000 men and 8.15 per 10,000 women per year.

About 2.5 million people are affected by this disease. There is clear cut geographical predisposition to South East Asia. Most of these cases are found in India although cases have been reported in Taiwan, Malaysia, South Africa, New Guniea,Sri lanka,Burma,United kingdom and Canada.[93]

Age :

There is no predilection for any age group however a broad age distribution with a peak in the range of 20-40 years was observed. [32]

Gender :

There is equal sex distribution, though some reports may vary indicating a female preponderance.[26]

Etiology :

The prime causes suspected on the basis of cause and effect relationship are prolonged and chronic use of arecanut, chillies, tobacco with arecanut, Pan masala,Pan ,Vitamin B complex deficiency.

Arecanut chewing has the strongest evidence regarding the etiology. Arecanut is the endosperm of the fruit areca catechu tree, the fruit of which is orange yellow in colour when ripe [71].

A 10 year prospective study on 10,000 subjects showed a zero incidence of oral submucous fibrosis among those who did not chew arecanut compared with an incidence of 35 per 10,000 among arecanut chewers.[10]

It has been shown that arecanut and its extract mainly arecoline can stimulate fibroblast proliferation, collagen synthesis and increasing collagen cross linking in in invitro studies. The flavinoids and tannins from betel nut can stabilize collagen and render them resistant to degradation by collagenase.[66]

Among systemic factors the main ones incriminated are chronic Iron, Vitamin B complex deficiency and anemia. Iron metabolism is important in maintaining the health of oral mucosa as well as the epithelium of the digestive tract and it contributes to normal enzyme activity.[26]

Clinical features :

The major presenting complaint is progressive inability to open the mouth owing to the accumulation of inelastic fibrous tissue in the juxtaepithelial region of the oral mucosa. Patients may describe a sudden onset of inflammation or ulceration of the oral mucosa and burning pain while eating highly seasoned food that previously caused no distress.[68]

The fibrosis also leads to difficulty in mastication speech and swallowing, pain in the throat and ears and relative loss of auditory acuity due to stenosis of the Eustachian tube. [68]

In the early cases the fibrosis is seen arching from the anterior pillars in to the soft palate as a delicate reticulum of interlacing white strands which later become confluent. In the cheeks a mottled marble like appearance may be seen when dense pale depigmented fibrosed areas alternate with pinker normal mucosa.[50, 67]

The floor of the mouth become pale and thickened, the tongue gets reduced in size and mobility; bands of encircling collagen distort lips.

At any stage in the disease the overlying epithelium may become the site of nonspecific ulceration, dysplastic change or malignant transformation. If fibrosis extends in to the esophagus the patient may experience progressive dysphagia and reduced esophageal mobility.[48, 49]

Patients with Oral sub mucous fibrosis often complains of sudden onset of inflammation or ulceration of oral mucosa with vesicle formation and increased sensitivity or burning sensation when eating spicy food that

are followed by trismus,increasing difficulty in mastication speech and swallowing.[64,65]

In advanced cases the jaws may be in separable and the total inelastic mucosa is forced against the buccal aspects of the teeth were sharp edges or restorations may cause ulcerations which become secondarily infected. The fibrosis progress in to the posterior part of the buccal mucosa, the anterior pillar of fauces and the soft palate including uvula. [48, 49,62]

Gupta P C and Dinesh Chandra in 1992 [30] suggested the Clinical Grading as follows,

The diagnosis of Oral submucous fibrosis is based on the positive history of areca nut chewing, Clinical and histopathological criteria. The severity of the disease can be graded based on the clinical grading system.

Grade I :

Presence of only blanching of oral mucosa with out symptoms,

Grade II :

Presence of blanching and burning sensation, dryness mouth, vesicles or ulcers in the mouth.

Grade III :

Presence of blanching and burning sensation, dryness mouth, vesicles or ulcers in the mouth with restriction of mouth opening and palpable bands all over the mouth with out tongue involvement.

Grade IV :

Presence of blanching and burning sensation, dryness mouth, vesicles or ulcers in the mouth with restriction of mouth opening and palpable bands all over the mouth without tongue involvement.

Grade V :

Presence of all features of Grade IV with tongue involvement.

Grade VI :

Oral sub mucous fibrosis along with histopathologically proven carcinoma.

Malignant transformation:

Paymaster in 1956 [56] was the first one to mention the precancerous nature of Oral submucous fibrosis when he observed the development of slowly growing Squamous cell carcinoma in One third of his Oral submucous fibrosis patients. There is 3-19% malignant transformation in Oral sub mucous fibrosis.

Pindborg J.J in 1966 [56] reported that Oral submucous fibrosis patients in India have higher occurrence of leukoplakia and carcinomas than those without this disease.

Murthi P.R et al in 1995 [48] conducted a follow-up study of 66 patients over a 17-year period, and found cancer developed in 7.6% of patients.

Pindborg J.J in 1997 [58] summarised the criteria in support of the precancerous nature of the disease as,

1. Higher prevalence of leukoplakia among Oral submucous fibrosis patients.
2. High frequency of epithelial dysplasia.
3. Concurrent finding of Oral submucous fibrosis in oral cancer patients.
4. Histological diagnosis of carcinoma without clinical suspicion of it and
5. Incidence of oralcancer patients with oral submucous fibrosis.

Differential diagnosis [44] :

1. Radiation induced fibrosis : which is confirmed by proper history taking regarding cancer history and radiation therapy.
2. Scleroderma: which is detected by the clinical features of the disease like progressive sclerosis all over the body, including skin and internal organs.
3. Fibrosis due to Actinomycosis infection : Patients history reveals period of infection and the treatment obtained.
4. Fibrosis due to Trauma : patient history reveals history of trauma, surgery.

Review on Oral Submucous Fibrosis:

Maher R et al in 1994 [42] conducted a case control study in Role of arecanut in the causation of Oral submucous fibrosis. Information on habits was collected by personal interview of 157 cases and 157 controls. Despite overall female preponderance, a substantial number of young men were enlisted. The male/female risks were found to be similar.

No differences between risks were found when comparing the three age categories, 0-20, 21-40, 41-60 yr. Among the cases, an increased risk was observed for areca nut chewing. This habit when practiced alone appeared to have the highest risk (Relative Risk 154), followed by pan with or without tobacco (Relative Risk 64, 32 respectively). Logistic regression and discriminant analysis showed that daily consumption rates appeared to be more important with respect to risk than lifetime duration of habit.

Tobacco habits were more prevalent amongst those 15 cases who presented with concurrent carcinoma and OSMF. They concluded that areca nut chewing has a causal relationship with OSMF. Additional tobacco insult may be necessary for subsequent carcinoma development.

Rajendran.R in 1994 [64] reviewed the etiology and pathogenesis of Oral submucous fibrosis. According to him oral sub mucous fibrosis, a pre cancerous condition of the oral cavity has been studied by a number of workers in the field. The available epidemiological data showed a clear cut geographical and ethnic pre dispositions, which suggested that certain customs or habits (chewing) prevalent among the population groups in

South East Asia might be possible etiological factors. However none of these customs was shown to be casually linked. This led some workers to consider the importance of systemic pre dispositions, in addition to the effects of local factors on the oral mucosa. More research is needed to elucidate this problem.

Shah N and Sharma R in 1994 [82] conducted Immunological studies in oral submucous fibrosis. He evaluated in 113 cases and 25 controls. The male/female ratio was 1.5/1. The mean age of males was significantly lower than that of females. The mean ESR levels were within normal limits, but for a higher than 20 mm fall per hr. in 40% of the cases. The serum IgA, IgG, and IgM levels were elevated significantly as compared to the controls. Circulating auto-antibodies and tissue-deposited antibodies were also found in 33% and 40% of the cases, respectively. From the analysis of the results, it is difficult to ascribe an auto-immune basis for the causation of OSMF. The female bias and elder age group, the factors generally considered in favour of an immune disorder, was not found in the study. However, raised ESR in 40% and serum globulin levels in 47% of the patients, distinctly higher levels of serum immunoglobulins, and positivity for circulating and tissue deposited antibodies in 33% and 34% of the cases respectively, do indicate an immunological basis.

Murti.P.R. in 1995 [48] reviewed the etiology of oral submucous fibrosis, a high risk pre cancerous condition, predominantly affecting Indians. Consumption of chilly was hypothesized as an etiologic factor on

the basis of ecological observations and a solitary animal experimental study. Subsequent epidemiologic studies that included case-series reports, large cross-sectional surveys, case control studies, cohort and intervention studies have identified arecanut as the major etiologic factor. Currently the role of genetic susceptibility and that of autoimmunity are receiving attention. Influence of nutritional factors if any remains unclear.

Gupta PC, et al in 1998 [32] conducted a study in India A total of 11,262 men and 10,590 women aged 15 years and older were interviewed for their tobacco habits. Among 5018 men who reported the use of tobacco or areca nut, 164 were diagnosed as suffering from OSF. All but four cases were diagnosed among 1786 current areca nut users (age-adjusted relative risk: 60.6). Areca nut was used mostly in mawa, a mixture of tobacco, lime and areca nut, and 10.9% of mawa users had OSF (age-adjusted relative risk: 75.6). The disease as well as areca nut use was concentrated (about 85%) in the lower (< 35 years) age group. They concluded the study by depicting an increase in the prevalence of OSF, especially in the lower age groups, directly attributable to the use of areca nut products. This could lead to an increase in the incidence of oral cancer in the future.

Shah N, Sharma PP in 1998 [83] conducted a study to identify the role of chewing and smoking habit in the etiology of oral submucous fibrosis. In this study 236 cases of oral submucous fibrosis were compared with 221 control subjects matched for age, sex and socioeconomic conditions. It was found that chewing of areca nut, quid and pan masala was directly related to

oral submucous fibrosis and not a single case was found without any chewing habit.

The study showed that the pan masala chewers develop oral submucous fibrosis in half the time taken by areca nut betel quid chewers. It was also found that duration of chewing was not significantly correlated but the frequency of chewing was directly correlated to manifestation of oral submucous fibrosis.

Zain RB et al in 1999 [102] reviewed Oral mucosal lesions association with betel quid, areca nut and tobacco chewing habits. A variety of betel/areca nut/tobacco habits have been reviewed and categorized because of their possible causal association with oral cancer and various oral precancerous lesions and conditions, and on account of their widespread occurrence in different parts of the world. He found that there is strong association between betel quid, arecanut, tobacco chewing habits and lesions such as chewer's mucosa, areca nut chewer's lesion, oral submucous fibrosis and other quid-related lesions.

Rajendran.R in 2003 [68]**,** reviewed the etiology, clinical features, epidemiology, pathology and management of Oral submucous fibrosis and concluded in his study as Oral submucous fibrosis is a chronic progressive, scaring disease that predominantly affects people of South East Asian origin.

Ranganathan K et al in 2004 [69] conducted a case control study in south India, over a 3 year period. A total of 185 consecutive patients with

OSF were matched with age- and sex-matched controls. History was recorded in a pre-determined format by qualified Dental Surgeons. The results obtained were , the male to female ratio of OSF cases was 9.9 : 1. All areca nut products were associated with OSF, with the risk being greatest for pan masala. The duration of the habit was more significant than the frequency of the chewing habit. The present study confirms the strong association between areca nut use and OSF and the increasing use of pan masala.

Punnya V et al in 2010, [61] made a a clinicopathologic study of 205 cases in Indians. The study evaluated 205 cases of oral submucous fibrosis for the age, sex, site of involvement, duration of disease at the time of diagnosis, associated habits and common presenting symptoms, presence of other mucosal lesions, malignant potential, and the histopathology.

The results revealed Oral submucous fibrosis seen in younger age (20–30 years) than that reported in literature and showed a characteristic male preponderance. A strong association with smokeless tobacco use especially arecanut in the form of gutkha was established and was related to earlier development of oral sub mucous fibrosis within a year of the habit. A total of 11.6% of cases were associated with malignancy and occurred predominantly in males.

CIRCULATING IMMUNE COMPLEXES:

Immune complexes are clusters of interlocking antigens and antibodies. Under normal conditions immune complexes are rapidly

removed from the bloodstream by macrophages in the spleen and Kupffer cells in the liver. In some circumstances, however, immune complexes continue to circulate.

Immune complexes may themselves cause disease when they are deposited in organs, e.g. in certain forms of vasculitis. This is the third form of hypersensitivity in the Gell-Coombs classification, called Type III hypersensitivity.

Immune complex deposition is a prominent feature of several autoimmune diseases, including systemic lupus erythematosus, cryoglobulinemia, rheumatoid arthritis, scleroderma and Sjögren's syndrome. [52, 72, 87]

Circulating immune complex disease occurs when the host's antibody production, relative to the amount of antigens, is inadequate for prompt elimination of antigen. Normally, excess amounts of antibody are formed which generate large immune complexes that are removed very rapidly from the circulation and are disposed of by the mononuclear phagocytic system. If the antibody response is very poor, only a few very small complexes are formed which are not prone to vascular deposition. [14]

When the relative antibody production is such that complexes of intermediate size form, vascular trapping can occur and injury results from the effects of inflammation. In addition to immune complex size, other factors influence vessel deposition, including the efficiency of systemic

clearance of immune complexes, the hemodynamics of blood flow, and vasoactive amine-influenced changes in vascular permeability.[78]

Intensive studies have documented the role of immune complexes as modulators of both cellular and humoral immune response. The occurrence of Circulating Immune Complexes as a marker for tumor burden and prognosis in the sera of patients with oral precancer and cancer is now well established. Recent advances in the fields of CIC, tumor progression, drug resistance, tumor cell heterogeneity and metastasis have resulted in a renewed interest in the development of non- specific immunotherapeutic modalities .[23,24]

The overall consensus is that only a small percentage of the detected CIC in vivo represent tumor associated antigens complexed with antibodies. The bulk of CIC most likely represent auto antibodies or the reaction to denatured self proteins, microbes, normal lymphocyte, antigens and nuclear antigens. Antigenic make up of CIC in cancer patients reflects the host's immune response to a variety of often overlapping antigenic stimuli and hence paves way for further studies.[82]

Immunological and biochemical alterations in the sera of such patients can help not only in early diagnosis, appropriate treatment but also as indicators of prognosis, as the disease progresses. CIC represent the host's physiological and immunological defense response in eliciting specific antibodies upon exposure to most antigenic substances. CIC deposition further leads to inflammation and tissue/ cell damage. It also

leads to suppression of cell mediated immunity and modulates the humoral responses.[78]

Circulating immune complexes are normally removed by the mononuclear phagocytic cells. However, circulating immune complexes formations or their defective clearance under certain circumstances becomes detrimental to the host, resulting in pathological deposition. Thus, altering the host immunological response leading to inflammation and tissue injury .

It can be suggested that immunological and biochemical assessment of markers such as CIC in oral precancer patients may help in earlier diagnosis and/or prognosis of these lesions. The CIC levels in Serum also help in predicting malignant potential of the pre malignant lesions.[38, 72,78, 95.]

Review on Circulating Immune Complexes:

Digeon et al in 1977 [20] investigated for elevated circulating immune complexes by precipitation tests using Poly Ethylene Glycol (PEG) on a series of normal and pathological sera. Various factors affecting PEG precipitation were studied. Immunoglobulins and complement factors precipitated by PEG (3.5%) were quantified and their significance was discussed in relation to serum levels. The PEG test was compared to labeled Complement 1q binding test with a fairly good correlation.

The direct evaluation of the amount of Complement 4 precipitated with IgG by 3% PEG provided a simpler routine assay than the C1q binding test for detecting complement-fixing immune complexes. The direct PEG test and the C4 test gave positive results in patients with diseases generally

presumed to be associated with immune complexes including Systemic lupus erythematosus, Acute glomerulonephritis, Sub-acute bacterial endocarditis and Chronic active hepatitis. The demonstration of Hepatitis B surface antigen and antibody after acid dissociation of PEG precipitates from Hepatitis B seronegative sera illustrated the fact that PEG does precipitate and thus concentrates circulating immune complexes.

Ríha et al in 1979 [74] used poly ethylene glycol for immune complex detection in human sera. Proteins precipitated from diluted sera of patients suffering from diseases with Circulating Immune Complexes by 3.75% Polyethyleneglycol (PEG) were studied. The low solubility at neutral pH and the good solubility at pH 8.4 with the finding of immunoglobulins and complement components in these precipitates and the presence of aggregates larger than 19S found by ultracentrifugation in positive sera are further proofs that under these conditions preferably IC are precipitated.'

These results revealed the conclusion that the 3.75% PEG precipitation of human sera diluted 1:30 in borate buffer, pH 8.4, is a reliable method for IC detection, especially useful for screening of large number of sera and for isolation of IC from positive sera.

Crispian Scully in 1983 [13] summarised in his study the evidence for the involvement of the immune response in the development of neoplasia, and discussed the immunological abnormalities found in patients with head and neck carcinoma, and outlined the recent attempts at treatment of patients

with head and neck carcinoma by modulation of the immune response which is also known as Immunotherapy.

Krapf F et al in 1983 [39] ,studied the elevated levels of circulating immune complexes in malignant diseases. By using three different assay methods, circulating immune complexes have been detected in 85% of sera from patients with malignant melanoma, and in 77% of sera from patients with breast cancer. These methods were a C1q-binding assay, a double-antibody conglutinin-binding ELISA, and a polyethylene glycol 6000 precipitation technique followed by quantitative determination of immunoglobulins in the redissolved precipitate.

Detection rates of circulating immune complexes using any one of these methods separately ranged from 33% to 56%, indicating the presence of different types of circulating immune complexes in cancer patients' sera. The combined use of the three methods mentioned resulted in an increased diagnostic sensitivity and a doubling of the predictive value [95]. However, tests for circulating immune complexes cannot be considered as useful parameters for early diagnosis of cancer, since the comparatively low incidence of malignancies in the population at large, together with the presence of circulating immune complexes in other, nonmalignant, diseases of considerable prevalence, appears to preclude effective application of any nonspecific method for early diagnosis of cancer in general.

Ostreiko K.K. et al in 1983 [53], used a method for rapid determination of average masses and concentrations of circulating immune

complexes in human sera is suggested. It is based on the dissimilarity in solubilities of immune complexes with different masses in the presence of polyethyleneglycol. Light-scattering intensities are measured by a laser nephelometer after adding to the serum of PEG in two different concentrations. The experimental values of the average masses and concentrations are calculated using calibration curves. The calibration curves are plotted for model immune complexes with different average masses obtained by heat-aggregation of IgG at various concentrations. This technique has been employed for determination of the average masses and concentrations of circulating immune complexes in 17 patients suffering from systemic lupus erythematosus and in 8 control individuals.they concluded theren exists significsnt correlation between curve values and CIC level in sera.

Daniel P. Eskinazi in 1984 [16] reviewed Advantages of sequential polyethylene glycol (PEG) precipitations to isolate and analyze circulating immune complexes. He reported a new approach utilizing sequential polyethylene glycol 6000 (PEG) precipitations to isolate and analyze circulating immune complexes (CIC). Two experimental systems were studied. He concluded that PEG concentrations needed to precipitate CIC cannot be predetermined, as they are a function of the size and probably the nature of the CIC studied. This limitation can be overcome by the systematic sequential precipitations which increase the chances of

identifying antigens and decrease the risks of confusing antigen-antibody complexes and Ig aggregates.

Endo L et al in 1985 [22] reviewed Clinical utility of assays for circulating immune complex. There are now many assays for the quantification of circulating immune complexes, each with distinct specificity and sensitivity. In a wide variety of rheumatic, infectious, neoplastic, and metabolic conditions, levels of circulating immune complexes may be elevated.

In selected situations, determination of circulating immune complex levels may help clinicians in the management of their patients. In lupus erythematosus, circulating immune complex levels, in conjunction with other immune parameters, may provide more insight into the disease course and activity than assessment of end organ parameters alone. In the differential diagnosis of infective endocarditis, serial levels of circulating immune complexes may provide evidence of effectiveness or failure of treatment. There is evidence that assays for circulating immune complexes may be of potential benefit in the management of Lyme disease and acute myelogenous leukemia.

Prabha Balaram et al in 1987 [59] made a study on Immunology of premalignant and malignant conditions of the oral cavity .Quantitation of circulating immune complexes (CIC) levels was established in patients with oral cancer and oral precancerous lesions. The levels were compared with that in normal controls and chronic chewers of betal quid with no signs of

any disease. Both patients with oral cancer and oral precancerous lesions had elevated CIC when compared to both the control groups. The most interesting observations were (a) the CIC levels in the chewing controls were significantly raised when compared to normal controls; and (b) the CIC levels in the patients with premalignant lesions were elevated almost to the same levels as in the oral cancer patients.

Mehar Aziz et al in 1992 [46] , made a study to detect CIC Child malignancy. Circulating immune complexes (CIC) were estimated in 28 cases of Non-Hodgkin's lymphomas, Hodgkin's disease, bone and soft tissue sarcomas in the pediatric age group by polyethylene glycol (PEG) precipitation and latex agglutination inhibition (LAI) techniques.

Results were comparted with 25 age matched controls. Highly significant CIC values were obtained by LAI technique ($P<0.01$) as compared to PEG precipitation technique ($P<0.05$) in malignancy. However, seropositivity for CIC in lymphomas and Hodgkin's disease was 85.71 per cent by LAI test as compared to 57.14 per cent by PEG pptn test. In sarcoma group, seropositivity for CIC was 57.11 per cent by LAI test and 28.57 per cent by PEG precipitation test.

Combination of both these tests increases the sensitivity of immune complex detection in serum of cancer patients. CIC begin to rise in serum in early stages of neoplastic transformation, and the level of CIC is directly proportional to proliferating tumor mass in vivo.

Vijay kumar et al in 1993 [98] reviewed Immunological phenomena in human oral carcinoma in India and concluded that there exists significant correlation between immunological alterations and degree of lesion from precancer to cancer. He emphasized that many oral cancers first present as a precancerous lesion and hence it is important to understand the biological factors associated with such lesions. The immune system and its functioning is a significant element in the development of cancer. Considerable evidence exists suggesting that the immune response plays a role in regulating the development and growth of oral precancers. He reviewed various immunological abnormalities associated with oral precancers including cell surface changes, alterations in cell mediated immune reactions and humoral immune abnormalities.

Makoto Kawata et al in 1998 [43] in his study detected Epithelial Ovarian-Cancer-Associated Antigens Involved in Immune Complexes by Monoclonal Antibodies. To detect antigenic molecules involved in immune complexes (ICs) in patients with epithelial ovarian cancer, they developed several monoclonal antibodies (Mabs) by hybridoma technology from mice immunized with ovarian cancer tissues. Hybridoma supernatants were differentially screened with a panel of ICs purified from ascitis of patients with ovarian cancer and with ICs from pooled normal human sera by the Enzyme-Linked Immuno Sorbent Assay (ELISA).

Furthermore, ICs detected, were elevated in the sera from 18 of 42 (42.9%) patients with epithelial ovarian cancer, but not in those from 39 patients with benign ovarian tumors or from 29 healthy individuals.

Sunali S Khanna et al in 2006 [91]evlauated the role of Circulating Immune Complexes and trace elements (Copper, Iron and Selenium) as markers in oral precancer and cancer in a randomised, controlled clinical trial. In serum of patients with Oral submucous fibrosis, oral leukoplakia, and Oral squamous cell carcinoma (OSCC), Circulating immune complexes (CIC) were estimated using 3.75% Polyethylene Glycol 6000(PEG) serum precipitation. Serum estimation of copper , Iron and selenium was done using the Oxalyl Dihydrazide method, Colorimetric Dipyridyl method and the Differential Pulse Cathodic Stripping Voltametry respectively.

The data analysis revealed increased circulating immune complex levels in the precancer and cancer patients. Among CIC, serum, copper, iron and selenium the best predictors for the occurrence of lesions were age, serum iron, CIC, serum selenium in the decreasing order.

Cynthia Jane et al in 2007 [15] investigated circulating immune complexes (CIC) as marker for disease progress in oral cancer.

The study comprised of 100 serum samples of 60 oral cancer patients having different grades of the disease and 40 patients with precancerous lesions were included. The results obtained were compared with those of group of 40 healthy blood donors. Modified PEG -6000 mediated precipitation was used to detect the level of CIC.

Elevated levels of Circulating Immune Complexes were observed in oral cancer patients and patients with oral precancerous lesions. 92% positive samples were observed in well differentiated squamous cell carcinoma whereas 100% positive samples were observed in both moderately and poorly differentiated squamous cell carcinoma. Oral leukoplakia and Oral submucous fibrosis showed 15% and 90% positivity respectively. Increased level of Circulating Immune complexes in high grade tumor suggest that Circulating Immune complexes are likely to contribute in evaluating the degree of malignancy.

Sameena Parveen et al in 2007 [78] conducted a follow-up study to evaluate the levels of circulating immune complexes and serum immunoglobulins in oral cancer patients The aim of the study was to estimate these immunological markers in pre- and post-treatment phases with a follow-up of 3-24 months and to understand the prognostic significance of the same in patients with oral cancer.

The malignancy group consisted of 56 patients with different stages of oral cancer and 20 healthy control group. Samples were selected at random and subjected for sequential analysis of serum biochemical markers (IgG, IgA, IgM and CIC-(Circulating immune complexes levels) in the pre- and post-treatment period. Statistical method employed was the paired t test. in results they observed significant elevated levels of all the immunological markers ($P < 0.01$) when compared with the control group.

Sequential analysis of these markers revealed significant reduction in immunological markers in stage I and II patients. On the contrary, stage III and IV patients showed remarkably elevated levels of IgA and CIC one year after initial treatment. All these immunological markers are indicative of tumor burden and Serum levels of CIC and IgA might be employed as prognostic indicators in oral cancer.

Sunali Khanna in 2008 [92] studied about Immunological and Biochemical Markers in Oral Carcinogenesis: The present study was designed to evaluate the immunologic and biochemical markers in oral carcinogenesis using circulating immune complexes (CIC), copper, iron, and selenium concentrations as assessment endpoints. Study results indicated an increase in CIC and copper levels, and a decrease in iron and selenium concentrations in oral cancer patients compared to controls $P < 0.0001$.

This is a Randomized Case control hospital based study conducted between April 2009 to May 2010 which was designed to estimate the Circulating immune complexes in serum in patients with potentially malignant lesion oral leukoplakia and potentially malignant condition Oral submucous fibrosis,in the department of Oral Medicine and Radiology of Ragas Dental College and Hospital, Uthandi, Chennai.

Study design:

The present study is a Randomised Case Control Study.

Study population:

Study population includes all subjects reporting to Ragas Dental college and Hospital, Outpatient Department, Uthandi seeking dental advice and who are from a wide variety of socioeconomic background. The age group selected was between 20-60 years.

Study sample:

A total number of 75 patients were involved in the study.

a) Patients with Oral leukoplakia-Group-I : 25
b) Patients with Oral submucous fibrosis –Group-II: 25
c) Normal controls-Group-III : 25

Obtaining approval from the authorities:

Permission from the ethical committee of **Ragas Dental College and Hospital**, Chennai was obtained before starting the study for interpretation and examining subjects, for drawing 5ml of blood .

Also an informed consent was obtained from the subjects forming the study sample, both in English and Tamil to participate in the study and to undergo blood investigation in the course of study.

Selection criteria:

For subjects with leukoplakia:

Inclusion criteria:

Routine intra oral examination was carried out on all subjects reporting to Ragas Dental College and Hospital ,Chennai and subjects with positive history of smoking tobacco and during oral soft tissue examination ,subjects with well-defined white patch,localized or extensive,that is slightly elevated and that has a fissured,wrinkled or corrugated surface or a mixed red – white lesion in which keratotic white nodules or patches are distributed over an atropic erythematous background or presence of thick white lesions with papillary surfaces in the oral cavity and on palpation which reveals leathery consistency and which is in consistant with the diagnosis of leukoplakia were taken for the study.

Exclusion criteria :

1. Lesions belonging to other entities such as Lichen planus,lupus erythematosus,leukedema and white sponge nevus and lesions for which etiology can be established, such as frictional keratosis, cheek/lip/tongue biting, contact lesions and stomatis nicotina palatini.
2. Subjects with major systemic ailments with cardiovascular, respiratory diseases were excluded.
3. Subjects with any form of immunosupression and with autoimmune disorders were excluded
4. Subjects with history of corticosteroid therapy were excluded.

For subjects with oral submucous fibrosis:

Inclusion criteria:

Routine intra oral examination was carried out on all subjects reporting to Ragas Dental College and Hospital ,Chennai ,subjects with positive history of arecanut chewing and during soft tissue examination clinical features like blanching of oral mucosa,burning sensation,dryness of mouth,vesicles or ulcers in the mouth ,restriction of mouth opening,palpable fibrotic bands in any area of the mouth with smooth and bald tongue with limited tongue movement and which is in consistent with the diagnosis of Oral submucous fibrosis were taken for the study.

Exclusion criteria :

1. Fibrosis due to radiation therapy, scleroderma, post Actinomycosis healing fibrosis, fibrosis due to trauma, surgery were excluded by taking proper case history and if positive history was present, they were excluded from the study.
2. Subjects with major systemic ailments with cardiovascular, respiratory diseases were excluded.
3. Subjects with any form of immunosupression and with autoimmune disorders were excluded
4. Subjects with history of corticosteroid therapy were excluded.

For Normal controls :

Inclusion criteria :

Routine intra oral examination was carried out on all subjects reporting to Ragas Dental College and Hospital ,Chennai and during soft tissue examination a through examination was carried out to rule out any mucosal lesions which is in consistent with the diagnosis of leukoplakia and Oral submucous fibrosis.

Exclusion criteria :

1. Subjects with major systemic ailments with cardiovascular, respiratory diseases were excluded.

2. Subjects with any form of immunosupression and with autoimmune disorders were excluded
3. Subjects with history of corticosteroid therapy were excluded.

Materials

Examination of the patient

- Conventional Dental chair with illumination facility with halogen lamp.
- A pair of sterile gloves.
- Disposable mouth mask.
- Stainless steel Kidney trays.
- Plain mouth mirror, straight probe, tweezer.
- Sterile gauze pieces and cotton.
- Glass tumbler with water.
- 0.2% chlorhexidine gluconate.
- Sterilizer, cheatel forceps.

Collection of blood sample:

- 24 gauge needle and 5ml plastic syringe
- Vacutainer coated with Ethylene diamine tetra acetic acid (EDTA)
- Torniquet
- Sterile Cotton

- 70% alcohol as surface disinfectant
- Sterile vials
- Refrigerator

Equipments:

- Centrifuge for separating plasma from blood
- Shimadzu UV spectrophotometer
- Micro pipette

The experimental subjects were made to sit comfortably on a dental chair. Relevant Demographic data and datas relevant to the habit of smoking, chewing and alcoholism were collected . Subjects were examined under halogen lamp .Sterile hand gloves were used during examination of the subjects .

For subjects who showed characteristic features of Oral leukoplakia and Oral submucous fibrosis based on history and clinical features , clinical diagnosis were made for each lesions separately.

The clinical features which is consistent with Oral leukoplakia in inspection and palpation are selected from the following features:

The site of leukoplakia depends on the type of smoking habit, the quality and the quantity of the tobacco. Most commonly involved sites are retro commissural area, buccal mucosa, edentulous alveolar ridge, hard

palate, tongue, lips. The gingival, soft palate and floor of mouth are less commonly involved .

Leukoplakia can be Homogeneous or Non homogeneous.

Inspectory findings of Homogeneous Leukoplakia reveals a thin, gray white plaques that may appear somewhat translucent, sometimes fissured or wrinkled .They usually have sharply demarcated borders but occasionally blend gradually in to normal mucosa.

In Non homogeneous Leukoplakia there may be few white coloured localized nodules or surface projections which gives rise to nodular Leukoplakia. Numerous white pointed projections developing on the surface gives an appearance of verruciform leukoplakia. When there is multiple oval or circular patches of redness in scattered areas that gives rise to Ulcerative Leukoplakia.

Palpatory findings reveals unscrapable white lesion which is leathery consistency and is non tender to palpate. The redness in the ulcerative Leukoplakia is non blanching when pressure is applied.

For Oral submucous fibrosis clinical diagnosis was made with the aid of clinical grading given by **Gupta P C and Dinesh Chandra in 1992 [30] .**

Grade I :

Presence of only blanching of oral mucosa with out symptoms,

Grade II :

Presence of blanching and burning sensation ,dryness mouth, vesicles or ulcers in the mouth .

Grade III :

Presence of blanching and burning sensation, dryness mouth, vesicles or ulcers in the mouth with restriction of mouth opening and palpable bands all over the mouth without tongue involvement.

Grade IV :

Presence of blanching and burning sensation, dryness mouth, vesicles or ulcers in the mouth with restriction of mouth opening and palpable bands all over the mouth without tongue involvement.

Grade V :

Presence of all features of Grade IV with tongue involvement.

Grade VI :

Oral submucous fibrosis along with histopathologically proven carcinoma.

Blood sample collection:

The patients arm was rested on the working table comfortably. The anti cubital fossa was exposed and the tourniquet was applied above 1 ½ - 2 inch above the anti cubital fossa. The area was rendered aseptic with 70% alcohol and using 24 gauge needles and vacationer 6 ml of blood was drawn, then the tourniquet was relieved and the needle was removed, simultaneously, a sterile cotton was placed on the needle puncture site and

instructions were given to apply finger pressure for 5 minutes and dispose the cotton. The collected blood was centrifuged, serum was separated and stored in vials . This freshly obtained serum was used immediately for biochemical analysis.

Biochemical Analysis

Estimation of Circulating Immune Complex in Serum :

Modified Poly Ethylene Glycol-6000(PEG) Mediated Precipation technique was used to estimate the levels of Circulating Immune Complex (CIC) in serum.[15]

Reagents:

1. 0.01M Borate buffer,with P^H-8.4
2. 4.166% Poly Ethylene Glycol.(PEG).

Procedure:

The serum will be aspirated and clarified by centrifugation at 1500g for 10min. Modified Poly Ethylene Glycol-6000(PEG) mediated precipation technique will be used to estimate the levels of Circulating Immune Complex (CIC) in serum. One part of the freshly obtained serum will be mixed with two parts of 0.01M Borate buffer,PH-8.4. To this mixture 27 parts of 4.166%PEG will be added to make the serum dilution into 1:30 with 3.75% PEG concentration.After incubation at room temperature for 60 minutes, the turbidity developed will be measured

spectrophotometrically 450nm against control containing 1:30 diluted serum in Borate buffer with out PEG. The level of CIC in serum will be expressed in terms of OD-450 measured at the end of 60 minutes.

Calculation:

$$\frac{\text{Optical density of the test}}{\text{Optical density of Standard}} \quad \text{X Concentration of Standard}$$

The optical density of the sample is expressed in Optical density 450nm. (OD 450 nm).

Statistical Analysis:

All the datas were entered in Microsoft excel sheets. Statistical analysis was done using SPSS software SYSTAT version 7.0.

Mean and standard deviation were estimated in the sample for each study group. Mean values were compared by using one-way ANOVA followed by multiple range tests by Tukey-HSD procedure.

In the present study P <0.05 was considered as the level of significance.

$$\text{Mean}(X) = \frac{\sum \overline{Xi}}{n}$$

$$\text{Standard Deviation} = \sqrt{\frac{\sum(\overline{Xi} - X)^2}{n-1}}$$

Where Xi is the individual observation and n is the sample size.

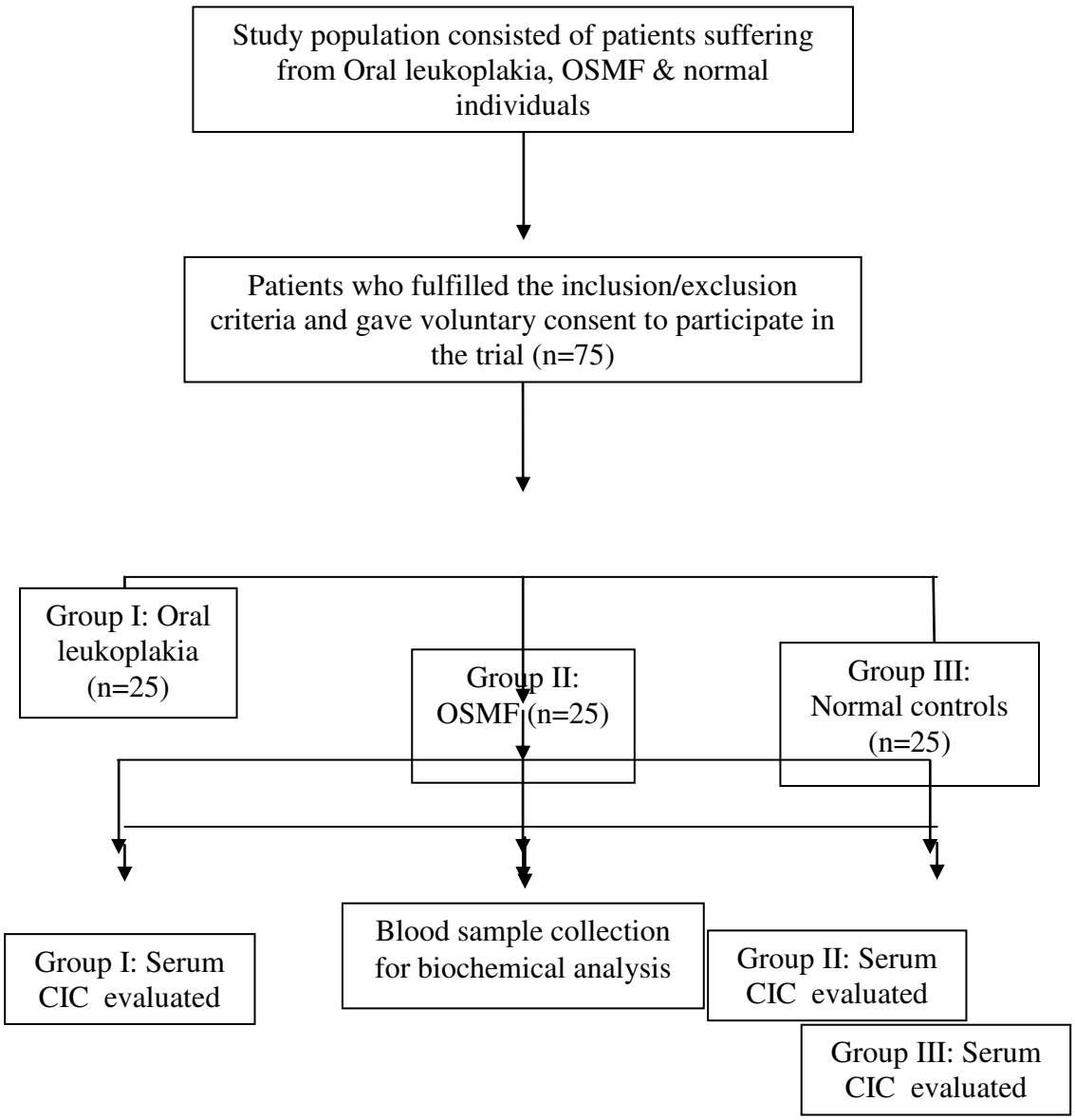
ANOVA:

$$\text{F Ratio} = \frac{\text{Variation between observed group averages}}{\text{Variation within each group}}$$

$$F = \frac{MS_{BETWEEN}}{MS_{WITHIN}}$$

$$\text{p value} = P(F_{k-l,n-k} > F \text{ obs})$$

STUDY OUTLINE



RAGAS DENTAL COLLEGE & HOSPITAL
2/102, EAST COAST ROAD, UTHANDI, CHENNAI – 600119
DEPARTMENT OF ORAL MEDICINE & RADIOLOGY

ESTIMATION OF CIRCULATING IMMUNE COMPLEXES IN PATIENTS WITH ORAL LEUKOPLAKIA AND ORAL SUBMUCOUS FIBROSIS -A CASE CONTROL STUDY.

Date:

S.No :

OP.No :

Study group : Group I / Group II / Group III

Name :

Age/Sex :

Address :

Phone number :

Occupation :

Monthly income :

Past medical /surgical/dental /history :

Chewing habits :

- Duration of chewing (<5yrs/ 5-10 yrs / 11-20 yrs / >21 yrs)

- Frequency of chewing per day (<5 times / 6-10times / >11times)

Smoking:

- Duration of smoking (<5yrs/ 5-10 yrs / 11-20 yrs / >21 yrs)
- Frequency of smoking per day (<5 times / 6-10times / >11times)

Alcohol consumption:

- Duration of alcohol consumption (<5yrs/5-10 yrs / 11-20 yrs / >21 yrs)
- Frequency of alcohol consumption per month (<5 times / 6-10times / >11times)

Leukoplakia :

Site :

Size :

Type :

Oral submucous fibrosis :

Grade :

Circulating Immune Complex level in OD 450 nm :

FIGURE 1. ARMAMENTARIUM FOR CLINICAL EXAMINATION

FIGURE 2. ARMAMENTARIUM FOR BLOOD INVESTIGATION

FIGURE 3.LABORATORY CENTRIFUGE MACHINE

FIGURE 4.SPECTROPHOTOMETER FOR ESTIMATION OF CIRCULATING IMMUNE COMPLEX

FIGURE 5. CHEMICAL REAGENTS

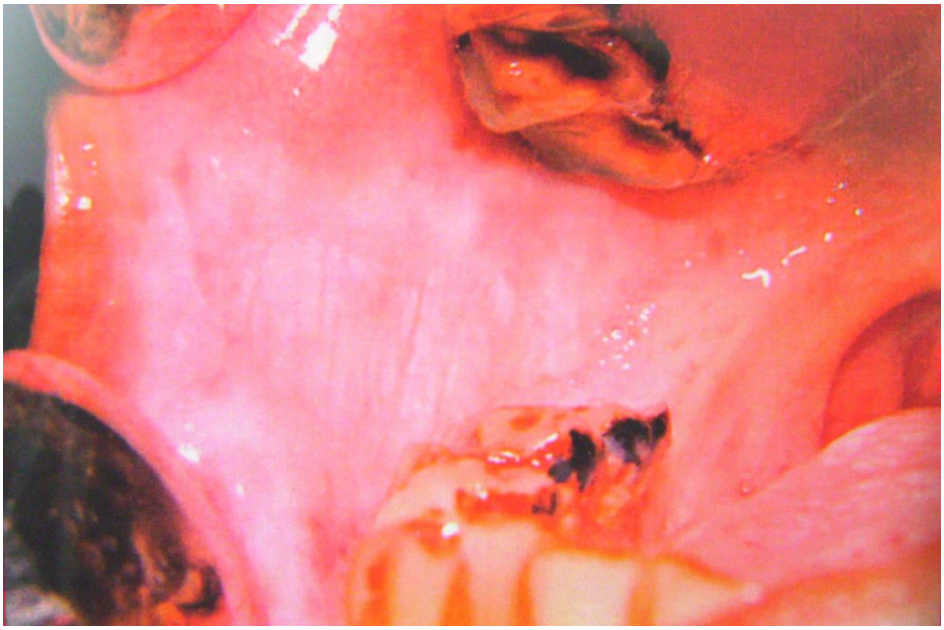
FIGURE 6. NORMAL ORAL MUCOSA

FIGURE 7.HOMOGENEOUS LEUKOPLAKIA

FIGURE 8.SPECKLED LEUKOPLAKIA

FIGURE 9.VERRUCOUS LEUKOPLAKIA

FIGURE 10.ORAL SUBMUCOUS FIBROSIS



The present study is a Randomized case control study which was conducted in the Department of Oral Medicine and Radiology of Ragas Dental College and Hospital,Uthandi, Chennai. It was devised to estimate the Circulating immune complexes in patients with Oral leukoplakia and Oral sub mucous fibrosis and healthy controls. The study was conducted between April 2009- May 2010 on a total of 75 subjects with 25 subjects in each group. The datas obtained from the study were statistically analysed. The results extracted are compared with various variables included in the study and are presented here.

Table – 1 Distribution of subjects in sex wise.

The study group consisted of a total number of 75 subjects. Out of the 75patients, 25 subjects were included in Oral leukoplakia (Group I) and among them 25(100%) were males and 0 (0%) were females, 25 were included in Oral submucous fibrosis (Group II) and among them 24 (96%) were males and 1(4%) females and 25 subjects were included in normal controls (Group III) and among them 22 (88%) were males and 3 (12%) were females.

The sex wise distribution of subjects were found to be **statistically non significant**, which means that both the experimental and control subjects were similar with respect to sex in distribution with **p value 0.157.**

Table – 2: Distribution of subjects in age wise.

The age of the subjects included in the study ranges between 20-60 years. So the subjects were divided into four age groups which are as follows: 20-30 years, 31-40 years, 41-50 years and 51-60 years . Among the 25 in group I, 06(24%) were between 20-30 years, 3(12%) were between 31-40 yrs , 12(48%) were between 41-50 years and 4(16%) were between 51-60 years. Among the 25 in group II, 15(60%) were between 20-30 years, 5(20%) were between 31-40 ,4(16%) were between 41-50 years and 1(4%) was between 51-60 years. Among the 25 in group III, 17(68%) were between 20-30 years, 03(12%) were between 31-40 ,2(8%) were between 41-50 years and 03(12%) were between 51-60 years.

The age wise distribution of subjects were found to be **statistically significant**,which means that there exists correlation among the 3 groups with respect to age in distribution with **p value 0.001**.

Table – 3: Distribution of subjects in age and sex wise in Group-I.

Table three shows the distribution of subjects based on age and sex in Group-I. The age range was from 23 to 60 years with the mean age of 41.92 years. There is a clear male predilection of 25(100%) compared to the females who accounted for 0(0%) in the total of 25.

Table – 4: Distribution of subjects in age and sex wise in Group-II.

Table four shows the distribution of subjects based on age and sex in Group II. The age range was from 22 to 55 years with the mean age of 32.76 years. There is a clear male predilection of 24(96%) compared to the females who accounted for 1(4%) in the total of 25.

Table – 5: Distribution of subjects in age and sex wise in Group-III.

Table five shows the distribution of subjects based on age and sex in the Group III. The age range was from 20 to 55 years with the mean age of 30.96 years. There is a clear male predilection of 22(88%) compared to the females who accounted for 3 (12%)in the total of 25.

Table – 6: Distribution of subjects based on habits in Group-I,Group-II and Group-III.

The distribution of habits were grouped as follows, chewing, smoking, alcohol, chewing and smoking, chewing and alcohol, smoking and alcohol and chewing, smoking and alcohol.

In Group I, 7(28%) had the habit of chewing, 25(100%) had habit of smoking , 11(44%) consumed alcohol, 7 (28%) had the habit of chewing and smoking ,5(20%) had the habits of chewing and alcohol consumption, 11(44%) had the habits of smoking and alcohol consumption and 5(20%) had all the three habits of chewing,smoking and alcohol consumption.

In group II, 25(100%) had the habit of chewing, 11(44%) had habit of smoking , 9(36%) consumed alcohol, 11 (44%) had the habit of chewing and smoking ,09(36%) had the habits of chewing and alcohol consumption ,6(24%) had the habits of smoking and alcohol consumption and 6(24%) had all the three habits of chewing,smoking and alcohol consumption.

In group III, 5(20%) had the habit of chewing, 5(20%) had habit of smoking , 3(12%) consumed alcohol.None had the habit of chewing and smoking , chewing and alcohol consumption , smoking and alcohol consumption and all the three habits of chewing,smoking and alcohol consumption.

The distribution of subjects based on habits were found to be **statistically significant**, with the habit of chewing , smoking, alcoholism alone or in combination of habits with the **p value 0.035**.

Table – 7: Distribution of subjects in age wise based on duration of smoking in Group-I.

The duration of smoking is subdivided into 5 categories, that is <5yrs,5-10yrs,11-15 yrs,16-20 yrs and >20yrs. In 20-30 years age group 1(4%) was with duration of smoking <5 times/day, 5(20%) were with duration of smoking 5-10 yrs . In 31-40 age group 3(12%) were with the duration of smoking 5-10 yrs. In 41-50 age group 5(20%) were with the duration of smoking 5-10yrs, 3(12%) were with the duration of smoking 11-15yrs ,3(12%) were with the duration of smoking 16-20yrs,1(4%) was with

the duration of smoking >20yrs. In 51-60 yrs 1(4%) was with the duration of smoking of 11-15yrs, and 3(12%) were with the duration of smoking >20 yrs.

Table – 8: Distribution of subjects in age wise based on frequency of smoking in Group-I.

The frequency of smoking is subdivided into three categories, that is < 5 times/day,5-10 times/day,11-15 times/day. In 20-30 years age group 6(24%) were with frequency of smoking 5-10 times/day. In 31-40 age group 3(12%) were with the frequency of smoking 5-10 times/day. In 41-50 age group 2(8%) were with the frequency of smoking <5times/day,10(40%) were with the frequency of smoking 5-10 times/day. In 51-60 age group 1(4%) was with the frequency of smoking < 5times/day,2(8%) were with the frequency of smoking 5-10 times/day and 1(4%) was with the frequency of smoking 11-15 times/day.

Table – 9: Distribution of subjects in age wise based on duration of smoking in Group-II .

The duration of smoking is subdivided into 5 categories, that is < 5yrs, 5-10yrs,11-15 yrs,16-20 yrs,>20yrs. In 20-30 years age group 4(16%) were with duration of smoking 5-10 yrs . In 31-40 age group 1(4%) was with the duration of smoking <5 yrs,2(8%) were with the duration of smoking 5-10 yrs. In 41-50 age group 3(12%) were with the duration of

smoking 5-10yrs,. In 51-60 yrs 1(4%) was with the duration of smoking of 5-10yrs.

Table – 10: Distribution of subjects in age wise based on frequency of smoking in Group-II.

The frequency of smoking is subdivided into three categories, that is < 5 times/day,5-10 times/day,11-15 times/day. In 20-30 years age group 2(8%) were with frequency of smoking < 5 times/day, 2(8%) were with frequency of smoking 5-10 times/day.. In 31-40 age group 1(4%) was with the frequency of smoking < 5 times/day, 2(8%) were with the frequency of smoking ,5-10 times/day. In 41-50 age group 2(8%) were with the frequency of smoking < 5times/day,1(4%) was with the frequency of smoking 5-10 times/day. In 51-60 age group 1(4%) was with the frequency of smoking < 5times/day.

Table – 11: Distribution of subjects in age wise based on duration of smoking in Group-III.

The duration of smoking is subdivided into 5 categories, that is < 5yrs,5-10yrs,11-15 yrs,16-20 yrs,>20yrs. In 20-30 years age group 2(8%) were with duration of smoking < 5 yrs ,1(4%) was with duration of smoking 5-10 yrs. In 31-40 age group 2(8%) were with the duration of smoking 5-10 yrs.

Table – 12: Distribution of subjects in age wise based on frequency of smoking in Group- III.

The frequency of smoking is subdivided into three categories, that is < 5 times/day,5-10 times/day,11-15 times/day. In 20-30 years age group 3(12%) were with frequency of smoking < 5 times/day. In 31-40 age group 2(8%) were with the frequency of smoking < 5 times /day.

Table -13 Distribution of smoking duration and frequency in Group-I,Group-II and Group-III.

In Group-I the No. of subjects with smoking habit were 25 with the mean duration 14.2 yrs with the minimum and the maximum duration ranges between 4 to 40 yrs.

In Group-II the No. of subjects with smoking habit were 11 with the mean duration 6.09 yrs with the minimum and the maximum duration ranges between 3 to 10 yrs.

In Group-III the No. of subjects with smoking habit were 5 with the mean duration 4.4 yrs with the minimum and the maximum duration ranges between 2 to 6 yrs.

In Group-I the No. of subjects with smoking habit were 25 with the mean frequency 7.16 times/day with the minimum and the maximum frequency ranges between 3 to 15 times/day.

In Group-II the No. of subjects with smoking habit were 11 with the mean frequency 3.64 times/day with the minimum and the maximum frequency ranges between 2 to 5 times/day

In Group-III the No. of subjects with smoking habit were 5 with the mean frequency 3.00 times/day with the minimum and the maximum frequency ranges between 2 to 4 times/day.

The habit of smoking has got high correlation among the three groups which is denoted by the p values for smoking duration 0.003 (Significant) and for smoking frequency :0.0002 (Significant).

Table – 14: Distribution of subjects in age wise based on duration of chewing in Group-I.

The duration of chewing is subdivided into 5 categories, that is <5yrs, 5-10yrs, 11-15 yrs,16-20 yrs,>20yrs. In 20-30 years age group 1(4%) was with duration of chewing < 5 yrs . In 31-40 age group 1(4%) was with the duration of chewing < 5 yrs. In 41-50 age group 4(16%) were with the duration of chewing < 5yrs,. In 51-60 yrs 1(4%) were with the duration of chewing of >20yrs.

Table – 15: Distribution of subjects in age wise based on frequency of chewing in Group-I .

The frequency of chewing is subdivided into three categories, that is < 5 times/day,5-10 times/day,11-15 times/day. In 20-30 years age group

1(4%) was with frequency of chewing 5-10 times/day. In 31-40 age group 1(4%) was with the frequency of chewing 5-10 times/day. In 41-50 age group 2(8%) were with the frequency of chewing < 5times/day,2(8%) were with the frequency of chewing 5-10 times/day. In 51-60 age group 1(4%) was with the frequency of chewing 5-10 times/day.

Table – 16: Distribution of subjects in age wise based on duration of chewing in Group-II.

The duration of chewing is subdivided into 5 categories, that is < 5yrs,5-10yrs,11-15 yrs,16-20 yrs,>20yrs. In 20-30 years age group 14(56%) were with duration of chewing 5-10 yrs , 1(4%) was with duration of chewing 11-15 yrs. In 31-40 age group 2(8%) were with the duration of chewing 5-10 yrs, 3(12%) were with the duration of chewing 11-15 yrs.. In 41-50 age group 1(4%) was with the duration of chewing 5-10yrs,, 2(8%) were with the duration of chewing 15-20yrs, 1(4%) was with the duration of chewing >20yrs. In 51-60 yrs 1(4%) was with the duration of chewing of >20yrs.

Table – 17: Distribution of subjects in age wise based on frequency of chewing in Group-II.

The frequency of chewing is subdivided into three categories, that is < 5 times/day,5-10 times/day,11-15 times/day. In 20-30 years age group 7(28%) were with frequency of chewing <5 times/day, 8(32%) were with

frequency of chewing 5-10 times/day.. In 31-40 age group 5(20%) were with the frequency of chewing < 5 times/day. In 41-50 age group 4(16%) were with the frequency of chewing < 5times/day. In 51-60 age group 1(4%) was with the frequency of chewing < 5 times/day.

Table – 18: Distribution of subjects in age wise based on duration of chewing in Group-III.

The duration of chewing is subdivided into 5 categories, that is <5yrs,5-10yrs,11-15 yrs,16-20 yrs,>20yrs. In 20-30 years age group 1(4%) was with duration of chewing < 5 yrs , 2(8%) were with duration of chewing 5-10 yrs. In 31-40 age group no chewing habit was found. In 41-50 age group 2(8%) were with the duration of chewing 5-10yrs. In 51-60 yrs no chewing habit was found.

Table – 19: Distribution of subjects in age wise based on frequency of chewing in Group-III.

The frequency of chewing is subdivided into three categories, that is < 5 times/day,5-10 times/day,11-15 times/day. In 20-30 years age group 3(12%) were with frequency of chewing < 5 times/day. In 31-40 age group no chewing habit was found.In 41-50 age group 2(8%) were with the frequency of chewing < 5 times/day. In 51-60 age group no chewing habit was found.

Table -20 Distribution of chewing duration and frequency in Group-I, Group-II and Group-III.

In Group-I the No. of subjects with chewing habit were 7 with the mean duration 6.43 yrs with the minimum and the maximum duration ranges between 2 to 25 yrs.

In Group-II the No. of subjects with chewing habit were 25 with the mean duration 11.24 yrs with the minimum and the maximum duration ranges between 5 to 30 yrs.

In Group-III the No. of subjects with chewing habit were 5 with the mean duration 4.8 yrs with the minimum and the maximum duration ranges between 4 to 5 yrs.

In Group-I the No. of subjects with chewing habit were 7 with the mean frequency 4.71 times/day with the minimum and the maximum frequency ranges between 2 to 6 times/day.

In Group-II the No. of subjects with chewing habit were 25 with the mean frequency 4.12 times/day with the minimum and the maximum frequency ranges between 2 to 7 times/day

In Group-III the No. of subjects with chewing habit were 5 with the mean frequency 2.40 times/day with the minimum and the maximum frequency ranges between 2 to 4 times/day.

The habit of chewing has got no correlation among the three groups with respect to chewing duration which is denoted by the p value 0.085 (not significant).

The habit of chewing has got correlation among the three groups with respect to chewing frequency which is denoted by the p value 0.030 (Significant).

Table – 21: Distribution of subjects in age wise based on duration of alcohol consumption in Group-I.

The duration of alcohol consumption is subdivided into 5 categories, that is < 5yrs,5-10yrs,11-15 yrs,16-20 yrs,>20yrs. In 20-30 and 31-40 years age group no habit of alcohol consumption were found.In 41-50 age group 7(28%) were with the duration of alcohol consumption 5-10yrs, 1(4%) was with the duration of alcohol consumption 16-20yrs.. In 51-60 yrs 3(12%) were with the duration of alcohol consumption of >20yrs.

Table – 22: Distribution of subjects in age wise based on frequency of alcohol consumption in Group-I.

The frequency of alcohol consumption is subdivided into three categories, that is < 5 times/month,5-10 times/month,11-15 times/month. In 20-30 years age group and in 31-40 age group no habit of alcohol consumption were found. In 41-50 age group 8(32%) were with the frequency of alcohol consumption < 5times/month. In 51-60 age group

1(4%) was with the frequency of alcohol consumption < 5 times/month, 2(8%) were with the frequency of alcohol consumption 5-10 times/month.

Table – 23: Distribution of subjects in age wise based on duration of alcohol consumption in Group-II.

The duration of alcohol consumption is subdivided into 5 categories, that is < 5yrs,5-10yrs,11-15 yrs,16-20 yrs,>20yrs. In 20-30 years age group 1(4%) was with the duration of alcohol consumption 5-10yrs,. In 31-40 age group 2(8%) were with the duration of alcohol consumption < 5yrs,, 2(8%) were with the duration of alcohol consumption 5-10yrs.. . In 41-50 age group 1(4%) was with the duration of alcohol consumption < 5yrs,, 2(8%) were with the duration of alcohol consumption 5-10yrs.. In 51-60 yrs 1(4%) was with the duration of alcohol consumption of 5-10yrs.

Table – 24: Distribution of subjects in age wise based on frequency of alcohol consumption in group II.

The frequency of alcohol consumption is subdivided into three categories, that is < 5 times/month,5-10 times/month,11-15 times/month. In 20-30 years age 1(4%) was with the frequency of alcohol consumption < 5times/month. In 31-40 years age 4(16%) were with the frequency of alcohol consumption < 5times/month. In 41-50 age group 3(12%) were with the frequency of alcohol consumption < 5times/day. In 51-60 age group 1(4%) were with the frequency of alcohol consumption <5

times/month, 2(8%) were with the frequency of alcohol consumption 5-10 times/month.

Table – 25: Distribution of subjects in age wise based on duration of alcohol consumption in Group-III.

The duration of alcohol consumption is subdivided into 5 categories, that is < 5yrs,5-10yrs,11-15 yrs,16-20 yrs,>20yrs. In 20-30 years age group no habit of alcohol consumption was found. 31-40 age group 1(4%) was with the duration of alcohol consumption < 5yrs. In 41-50 age group no habit of alcohol consumption was found.. In 51-60 yrs 2(8%) were with the duration of alcohol consumption of < 5yrs.

Table – 26: Distribution of subjects in age wise based on frequency of alcohol consumption in Group-III.

The frequency of alcohol consumption is subdivided into three categories, that is < 5 times/month,5-10 times/month,11-15 times/month. In 20-30 years age no habit of alcohol consumption was found. In 31-40 years age 1(4%) was with the frequency of alcohol consumption < 5times/month. In 41-50 age group no habit of alcohol consumption was found. In 51-60 age group 2(8%) were with the frequency of alcohol consumption < s5 times/month.

Table -27 Distribution of alcohol consumption duration and frequency in Group-I, Group-II and Group-III.

In Group-I the No. of subjects with alcohol consumption habit were 11 with the mean duration 15.73 yrs with the minimum and the maximum duration ranges between 5 to 40 yrs.

In Group-II the No. of subjects with alcohol consumption habit were 9 with the mean duration 6.89 yrs with the minimum and the maximum duration ranges between 4 to 10 yrs.

In Group-III the No. of subjects with alcohol consumption habit were 3 with the mean duration 13.33 yrs with the minimum and the maximum duration ranges between 5 to 20 yrs.

In Group-I the No. of subjects with alcohol consumption habit were 11 with the mean frequency 4.36 times/month with the minimum and the maximum frequency ranges between 1 to 10 times/month.

In Group-II the No. of subjects with alcohol consumption habit were 9 with the mean frequency 2.78 times/month with the minimum and the maximum frequency ranges between 2 to 4 times/month

In Group-III the No. of subjects with alcohol consumption habit were 3 with the mean frequency 2.33 times/month with the minimum and the maximum frequency ranges between 1 to 4 times/month.

The habit of alcohol consumption frequency has got no significant correlation among the three groups which means that Group-I,Group-II and Group-III were similar in alcohol consumption duration and frequency,

which are denoted by the p value for alcohol consumption duration the p value 0.098 (Not significant) and alcohol consumption frequency :0.235 (Not Significant) .

Table-28.Distribution of subjects in Group-I according to site of leukoplakia.

Among the 25 subjects in Group-I, 14 (56%) had lesion on reterocommisural area, 9(36%) had lesion in the buccal mucosa,1(4%) had lesion in the floor of the mouth,1(4%) had lesion in the tongue.

Table-29. Distribution of subjects in Group-I according to size of leukoplakia.

Among the 25 subjects in Group-I, the size of the lesion was less than 2cm in 7(28%) subjects,2-4 cm in 15 (60%) subjects and greater than 4cm in 3(12%) subjects.

Table-30. Distribution of subjects in Group-I according to type of leukoplakia.

Among the 25 subjects in Group-I, 19(76%) subjects had homogenous leukoplakia and 5(20%) subjects had speckled leukoplakia , 1(4%) subject had verrucous leukoplakia .None of the subjects had ulcerative leukoplakia.

Table-31. Distribution of subjects in Group-II according to clinical grade.

Among the 25 subjects in Group-II ,2(8%) had Clinical Grade of I,20(80%) had Clinical Grade of III,3(12%) had Clinical Grade of IV. None of the subjects had Clinical Grade of II.

Table 32.Circulating Immune Complex in Group-I,Group-II,Group-III.

The mean circulating immune complex value is highest in Oral submucous fibrosis subjects (Group-II)which is 0.11620,followed by oral leukoplakia subjects (Group-I) which is 0.05988 and the lowest value is seen with control subjects (Group-III) which is 0.02956. The p value in relation to the dependent variable Circulating immune complex with reference to Group-I,Group-II,Group-III is 0.0001 and it is significant.

Table 33.Mean,Standard deviation, test of significance of CIC in relation to Group-I, Group-II,Group-III.

The mean Circulating immune complex value in Group-I is 0.05988, and the minimum and the maximum values are 0.014 and 0.089 respectively with the standard deviation 0.024761.

The mean Circulating immune complex value in Group-II is 0.11620.The minimum and the maximum values are 0.048 and 0.251 respectively with the standard deviation 0.046957.

The mean Circulating immune complex value in Group-III is 0.02956. The minimum and the maximum values are 0.012 and 0.045 respectively with the standard deviation 0.008912.

The mean Circulating immune complex value in all the three Groups together, is 0.06855,with the minimum and the maximum values are 0.012 and 0.251 respectively with the standard deviation 0.047390.

P value = 0.0001 (Significant)

Table 34.Test of significance between Group-I, Group-II,Group-III in relation to the dependent variable : Circulating Immune Complex.

The mean difference between Group-I and Group-II is 0.056320, with p value 0.0001 which is significant. The mean difference between Group-I and Group-III is 0.030320, with p value 0.003 which is significant.

The mean difference between Group-II and Group-I is 0.056320, with p value 0.0001 which is significant. The mean difference between Group-II and Group-III is 0.086640, with p value 0.0001 which is significant.

The mean difference between Group-III and Group-I is 0.030320, with p value 0.003 which is significant. The mean difference between Group-II and Group-III is 0.086640, with p value 0.0001 which is significant.

TABLE-1: DISTRIBUTION OF SUBJECTS IN SEX WISE.

Sex	Group-I		Group-II		Group-III		Total	
Male	25	100%	24	96%	22	88%	71	94.7%
Female	0	0%	1	4%	3	12%	4	5.3%
Total	25	100%	25	100%	25	100%	75	100%

p value = 0.157 (not significant)

TABLE – 2: DISTRIBUTION OF SUBJECTS IN AGE WISE .

Age (yrs)	Group I		Group II		Group III		Total	
20-30	6	24%	15	60%	17	68%	38	50.6%
31-40	3	12%	5	20%	3	12%	11	14.6%
41-50	12	48%	4	16%	2	08%	18	24%
51-60	4	16%	1	04%	3	12%	8	10.6%
Total	25	100%	25	100%	25	100%	75	100%

p value = 0.001 (significant)

TABLE – 3: DISTRIBUTION OF SUBJECTS IN AGE AND SEX WISE IN GROUP-I.

SL.NO.	AGE	SEX
1.	48	M
2.	43	M
3.	40	M
4.	26	M
5.	42	M
6.	57	M
7.	57	M
8.	27	M
9.	60	M
10.	59	M
11.	43	M
12.	39	M
13.	43	M
14.	26	M
15.	37	M
16.	50	M
17.	45	M
18.	49	M
19.	23	M
20.	28	M
21.	44	M
22.	41	M
23.	47	M
24.	50	M
25	24	M

Age range : 23-60 Years Mean age – 41.92 Years

TABLE – 4: DISTRIBUTION OF SUBJECTS IN AGE AND SEX WISE IN GROUP-II.

SL.NO.	AGE	SEX
1.	27	M
2.	24	M
3.	30	F
4.	43	M
5.	24	M
6.	24	M
7.	24	M
8.	40	M
9.	29	M
10.	39	M
11.	47	M
12.	32	M
13.	29	M
14.	47	M
15.	24	M
16.	28	M
17.	30	M
18.	24	M
19.	26	M
20.	29	M
21.	32	M
22.	55	M
23.	40	M
24.	22	M
25	50	M

Age range : 22-55 Years. Mean age:32.76 Years

TABLE – 5: DISTRIBUTION OF SUBJECTS IN AGE AND SEX WISE IN GROUP-III.

SL.NO.	AGE	SEX
1.	52	M
2.	20	M
3.	25	M
4.	30	F
5.	30	M
6.	25	M
7.	23	M
8.	27	F
9.	37	M
10.	22	M
11.	29	M
12.	44	M
13.	20	M
14.	35	M
15.	21	M
16.	52	M
17.	30	M
18.	43	M
19.	27	M
20.	22	M
21.	25	M
22.	32	M
23.	55	M
24.	27	M
25	21	F

Age range : 20-55 Years Mean age:30.96 Years

TABLE – 6: DISTRIBUTION OF SUBJECTS BASED ON HABITS IN GROUP-I, GROUP-II AND GROUP-III.

Group	Chewing		Smoking		Alcohol		Chewing + Smoking		Chewing + Alcohol		Smoking + Alcohol		Chewing + Smoking + Alcohol	
Group I -	7	28%	25	100%	11	44%	7	28%	5	20%	11	44%	5	20%
Group II -	25	100%	11	44%	9	36%	11	44%	9	36%	6	24%	6	24%
Group III-	5	20%	5	20%	3	12%	0	0	0	0	0	0	0	0
GROUP I + GROUP II +GROUP III	37	49.3%	41	54.6%	23	30.6%	18	24%	14	18.6%	17	22.6%	11	14.6%

p value 0.035 (significant)

TABLE – 7 : DISTRIBUTION OF SUBJECTS IN AGE WISE BASED ON DURATION OF SMOKING IN GROUP-I.

Age (Yrs)	< 5 Yrs		5-10 Yrs		11-15 Yrs		16-20 Yrs		>20 Yrs	
20-30	1	4%	05	20%	0	0	0	0	0	0
31-40	0	0	3	12%	0	0	0	0	0	0
41-50	0	0	5	20%	3	12%	3	12%	1	4%
51-60	0	0	0	0	1	4%	0	0	3	12%
Total- 25	1	4%	13	52%	4	16%	3	12%	4	16%

TABLE – 8: DISTRIBUTION OF SUBJECTS IN AGE WISE BASED ON FREQUENCY OF SMOKING IN GROUP-I.

Age(Yrs)	< 5Times/day		5-10 Times/day		11-15 Times/day	
20-30	0	0	6	24%	0	0
31-40	0	0	3	12%	0	0
41-50	2	8%	10	40%	0	0
51-60	1	4%	2	8%	1	4%
Total- 25	3	12%	21	84%	1	4%

TABLE – 9 : DISTRIBUTION OF SUBJECTS IN AGE WISE BASED ON DURATION OF SMOKING IN GROUP-II.

Age (Yrs)	< 5 Yrs		5-10 Yrs		11-15 Yrs		16-20 Yrs		>20 Yrs	
20-30	0	0	4	16%	0	0	0	0	0	0
31-40	1	4%	2	8%	0	0	0	0	0	0
41-50	0	0	3	12%	0	0	0	0	0	0
51-60	0	0	1	4%	0	0	0	0	0	0
Total-25	1	4%	10	40%	0	0	0	0	0	0

TABLE – 10 : DISTRIBUTION OF SUBJECTS IN AGE WISE BASED ON FREQUENCY OF SMOKING IN GROUP-II.

Age (Yrs)	< 5Times/day		5-10 Times/day		11-15 Times/day	
20-30	2	8%	2	8%	0	0
31-40	1	4%	2	8%	0	0
41-50	2	8%	1	4%	0	0
51-60	1	4%	0	0	0	0
Total -25	6	24%	5	20%	0	0

TABLE – 11: DISTRIBUTION OF SUBJECTS IN AGE WISE BASED ON DURATION OF SMOKING IN GROUP-III.

Age (Yrs)	< 5 Yrs		5-10 Yrs		11-15 Yrs		16-20 Yrs		>20 Yrs	
20-30	2	8%	1	4%	0	0	0	0	0	0
31-40	0	0	2	8%	0	0	0	0	0	0
41-50	0	0	0	0	0	0	0	0	0	0
51-60	0	0	0	0	0	0	0	0	0	0
Total-25	2	8%	3	12%	0	0	0	0	0	0

TABLE – 12: DISTRIBUTION OF SUBJECTS IN AGE WISE BASED ON FREQUENCY OF SMOKING IN GROUP-III.

Age (Yrs)	< 5Times/day		5-10 Times/day		11-15 Times/day	
20-30	3	12%	0	0	0	0
31-40	2	8%	0	0	0	0
41-50	0	0	0	0	0	0
51-60	0	0	0	0	0	0
Total - 25	5	20%	0	0	0	0

TABLE 13.DISTRIBUTION OF SMOKING DURATION AND FREQUENCY IN GROUP-I,GROUP-II AND GROUP-III.

Smoking	No. of subjects	Mean	Minimum	Maximum
Smoking duration in years				
Group-I	25	14.20	4	40
Group-II	11	6.09	3	10
Group-III	5	4.40	2	6
Total	41	10.83	2	40
Smoking frequency No. of times/day				
Group-I	25	7.16	3	15
Group-II	11	3.64	2	5
Group-III	5	3.00	2	4
Total	41	5.71	2	15

p value :

For smoking duration : 0.003 (significant)

For smoking frequency : 0.0002 (significant)

TABLE – 14: DISTRIBUTION OF SUBJECTS IN AGE WISE BASED ON DURATION OF CHEWING IN GROUP-I.

Age (Yrs)	< 5 Yrs		5-10 Yrs		11-15 Yrs		16-20 Yrs		> 20 Yrs	
20-30	1	4%	0	0	0	0	0	0	0	0
31-40	1	4%	0	0	0	0	0	0	0	0
41-50	4	16%	0	0	0	0	0	0	0	0
51-60	0	0	0	0	0	0	0	0	1	4%
Total-25	6	24%	0	0	0	0	0	0	1	4%

TABLE – 15: DISTRIBUTION OF SUBJECTS IN AGE WISE BASED ON FREQUENCY OF CHEWING IN GROUP-I.

Age (Yrs)	< 5Times/day		5-10 Times/day		11-15 Times/day	
20-30	0	0	1	4%	0	0
31-40	0	0	1	4%	0	0
41-50	2	8%	2	8%	0	0
51-60	0	0	1	4%	0	0
Total-25	2	8%	5	20%	0	0

TABLE – 16. DISTRIBUTION OF SUBJECTS IN AGE WISE BASED ON DURATION OF CHEWING IN GROUP-II.

Age (Yrs)	< 5 Yrs		5-10 Yrs		11-15 Yrs		16-20 Yrs		>20 Yrs	
20-30	0	0	14	56%	1	4%	0	0	0	0
31-40	0	0	2	8%	3	12%	0	0	0	0
41-50	0	0	1	4%	0	0	2	8%	1	4%
51-60	0	0	0	0	0	0	0	0	1	4%
Total - 25	0	0	17	68%	4	16%	2	8%	2	8%

TABLE – 17. DISTRIBUTION OF SUBJECTS IN AGE WISE BASED ON FREQUENCY OF CHEWING IN GROUP-II.

Age (Yrs)	< 5Times/day		5-10 Times/day		11-15 Times/day	
20-30	7	28%	8	32%	0	0
31-40	5	20%	0	0	0	0
41-50	4	16%	0	0	0	0
51-60	1	4%	0	0	0	0
Total - 25	17	68%	8	32%	0	0

TABLE – 18. DISTRIBUTION OF SUBJECTS IN AGE WISE BASED ON DURATION OF CHEWING IN GROUP-III.

Age (Yrs)	< 5 Yrs		5-10 Yrs		11-15 Yrs		16-20 Yrs		> 20 Yrs	
20-30	1	4%	2	8%	0	0	0	0	0	0
31-40	0	0	0	0	0	0	0	0	0	0
41-50	0	0	2	8%	0	0	0	0	0	0
51-60	0	0	0	0	0	0	0	0	0	0
Total -25	1	4%	4	16%	0	0	0	0	0	0

TABLE – 19. DISTRIBUTION OF SUBJECTS IN AGE WISE BASED ON FREQUENCY OF CHEWING IN GROUP-III.

Age (Yrs)	< 5Times/day		5-10 Times/day		11-15 Times/day	
20-30	3	12%	0	0	0	0
31-40	0	0	0	0	0	0
41-50	2	8%	0	0	0	0
51-60	0	0	0	0	0	0
Total -25	5	20%	0	0	0	0

TABLE 20.DISTRIBUTION OF CHEWING DURATION AND FREQUENCY IN GROUP-I,GROUP-II AND GROUP-III.

Chewing	No. of subjects	Mean	Minimum	Maximum
Chewing duration In years				
Group-I	7	6.43	2	25
Group-II	25	11.24	5	30
Group-III	5	4.80	4	5
Total	37	9.46	2	30
Chewing frequency times/day				
Group-I	7	4.71	2	6
Group-II	25	4.12	2	7
Group-III	5	2.40	2	4
Total	37	4.00	2	7

p value :
For chewing duration : 0.085(not significant)
For chewing frequency : 0.030 (significant)

TABLE – 21. DISTRIBUTION OF SUBJECTS IN AGE WISE BASED ON DURATION OF ALCOHOL CONSUMPTION IN GROUP-I.

Age (Yrs)	< 5 Yrs		5-10 Yrs		11-15 Yrs		16-20 Yrs		> 20 Yrs	
20-30	0	0	0	0	0	0	0	0	0	0
31-40	0	0	0	0	0	0	0	0	0	0
41-50	0	0	7	28%	0	0	1	4%	0	0
51-60	0	0	0	0	0	0	0	0	3	12%
Total-25	0	0	7	28%	0	0	1	4%	3	12%

TABLE – 22. DISTRIBUTION OF SUBJECTS IN AGE WISE BASED ON FREQUENCY OF ALCOHOL CONSUMPTION IN GROUP-I.

Age (Yrs)	< 5 Times/month		5-10 Times/month		11-15 Times/month	
20-30	0	0	0	0	0	0
31-40	0	0	0	0	0	0
41-50	8	32%	0	0	0	0
51-60	1	4%	2	8%	0	0
Total -25	9	36%	2	8%	0	0

TABLE – 23. DISTRIBUTION OF SUBJECTS IN AGE WISE BASED ON DURATION OF ALCOHOL CONSUMPTION IN GROUP-II.

Age (Yrs)	< 5 Yrs		5-10 Yrs		11-15 Yrs		16-20 Yrs		> 20 Yrs	
20-30	0	0	1	4%	0	0	0	0	0	0
31-40	2	8%	2	8%	0	0	0	0	0	0
41-50	1	4%	2	8%	0	0	0	0	0	0
51-60	0	0	1	4%	0	0	0	0	0	0
Total-25	3	12%	6	24%	0	0	0	0	0	0

TABLE – 24. DISTRIBUTION OF SUBJECTS IN AGE WISE BASED ON FREQUENCY OF ALCOHOL CONSUMPTION IN GROUP-II.

Age(Yrs)	< 5 Times/month		5-10 Times/month		11-15Times/month	
20-30	1	4%	0	0	0	0
31-40	4	16%	0	0	0	0
41-50	3	12%	0	0	0	0
51-60	1	4%	2	8%	0	0
Total-25	9	36%	2	8%	0	0

TABLE – 25. DISTRIBUTION OF SUBJECTS IN AGE WISE BASED ON DURATION OF ALCOHOL CONSUMPTION IN GROUP-III.

Age (Yrs)	< 5 Yrs		5-10 Yrs		11-15 Yrs		16-20 Yrs		>20 Yrs	
20-30	0	0	0	0	0	0	0	0	0	0
31-40	1	4%	0	0	0	0	0	0	0	0
41-50	0	0	0	0	0	0	0	0	0	0
51-60	2	8%	0	0	0	0	0	0	0	0
Total-25	3	12%	0	0	0	0	0	0	0	0

TABLE – 26. DISTRIBUTION OF SUBJECTS IN AGE WISE BASED ON FREQUENCY OF ALCOHOL CONSUMPTION IN GROUP-III.

Age (Yrs)	< 5Times/month		5-10 Times/month		11-15Times/month	
20-30	0	0	0	0	0	0
31-40	1	4%	0	0	0	0
41-50	0	0	0	0	0	0
51-60	2	8%	0	0	0	0
Total-25	0	0	0	0	0	0

TABLE 27.DISTRIBUTION OF ALCOHOL CONSUMPTION FREQUENCY AND DURATION IN GROUP-I,GROUP-II AND GROUP-III.

Alcoholism	No. of subjects	Mean	Minimum	Maximum
Alcohol duration In years				
Group-I	11	15.73	5	40
Group-II	9	6.89	4	10
Group-III	3	13.33	5	20
Total	23	11.96	4	40
Alcohol frequency times/month				
Group-I	11	4.36	1	10
Group-II	9	2.78	2	4
Group-III	3	2.33	1	4
Total	23	3.48	1	10

p value :

For alcohol duration : 0.098(not significant)

For alcohol frequency : 0.235 (not significant)

TABLE 28 .DISTRIBUTION OF SUBJECTS IN GROUP-I ACCORDING TO THE SITE OF LEUKOPLAKIA.

Sl.no	Site of leukoplakia	No.of subjects	Percentage
1.	Retro commisure	14	56 %
2.	Buccal mucosa	9	36%
3.	Floor of the mouth	1	4%
4.	Tongue	1	4%
	Total	25	100%

TABLE -29.DISTRIBUTION OF SUBJECTS IN GROUP-I ACCORDING TO THE SIZE OF LEUKOPLAKIA.

Sl.no	Size of leukoplakia	No. Of subjects	Percentage
1.	<2CM	7	28%
2.	2-4CM	15	60%
3.	>4CM	3	12%
	Total	25	100%

TABLE -30.DISTRIBUTION OF SUBJECTS IN GROUP-I ACCORDING TO THE TYPE OF LEUKOPLAKIA.

Sl no	Type	No. of subjects	Percentage
1.	Homogeneous leukoplakia	19	76%
2.	Ulcerative leukoplakia	0	0
3.	Speckled leukoplakia	5	20%
4.	Verrucous leukoplakia	1	4%
		Total	100%

TABLE -31 DISTRIBUTION OF SUBJECTS IN GROUP II ACCORDING TO CLINICAL GRADE.

Sl.no	Clinical grade	No. Of subjects	Percentage
1.	I	2	8%
2.	II	0	0
3.	III	20	80%
4.	IV	3	12%
	Total	25	100%

TABLE 32.CIRCULATING IMMUNE COMPLEX IN GROUP-I,GROUP-II AND GROUP-III.

Sl.no	GROUP-I CIC vaule in OD-450nm	GROUP-II CIC vaule in OD-450nm	GROUP-III CIC vaule in OD-450nm
1.	0.025	0.222	0.025
2.	0.015	0.048	0.012
3.	0.014	0.084	0.034
4.	0.025	0.251	0.023
5.	0.063	0.118	0.024
6.	0.053	0.101	0.030
7.	0.078	0.098	0.027
8.	0.080	0.099	0.033
9.	0.071	0.089	0.022
10.	0.081	0.112	0.012
11.	0.072	0.116	0.041
12.	0.065	0.222	0.034
13.	0.021	0.099	0.033
14.	0.053	0.125	0.028
15.	0.080	0.125	0.035
16.	0.077	0.092	0.032
17.	0.062	0.123	0.025
18.	0.052	0.092	0.045
19.	0.082	0.084	0.043
20.	0.074	0.098	0.017
21.	0.087	0.092	0.031
22.	0.088	0.098	0.035
23.	0.089	0.124	0.029
24.	0.025	0.085	0.045
25.	0.065	0.108	0.024

Mean values in OD 450 nm for
Group I : 0.05988
Group II :0.11620
Group III : 0.02956 p value : 0.0001 (Significant)

TABLE 33.MEAN,STANDARD DEVIATION,TEST OF SIGNIFICANCE OF CIC IN RELATION TO GROUP-I,GROUP-II AND GROUP-III.

Group	No. Of subjects	Mean CIC values in OD 450nm	Standard deviation	Minimum CIC value in OD 450 nm	Maximum CIC value in OD 450 nm
GROUP-I	25	0.05988	0.024761	0.014	0.089
GROUP-II	25	0.11620	0.046957	0.048	0.251
GROUP-III	25	0.02956	0.008912	0.012	0.045
TOTAL	75	0.06855	0.047390	0.012	0.251

P value :0.0001(significant)

TABLE 34.TEST OF SIGNIFICANCE BETWEEN GROUP-I,GROUP-II,GROUP-III IN RELATION TO THE DEPENDENT VARIABLE – CIRCULATING IMMUNE COMPLEX.

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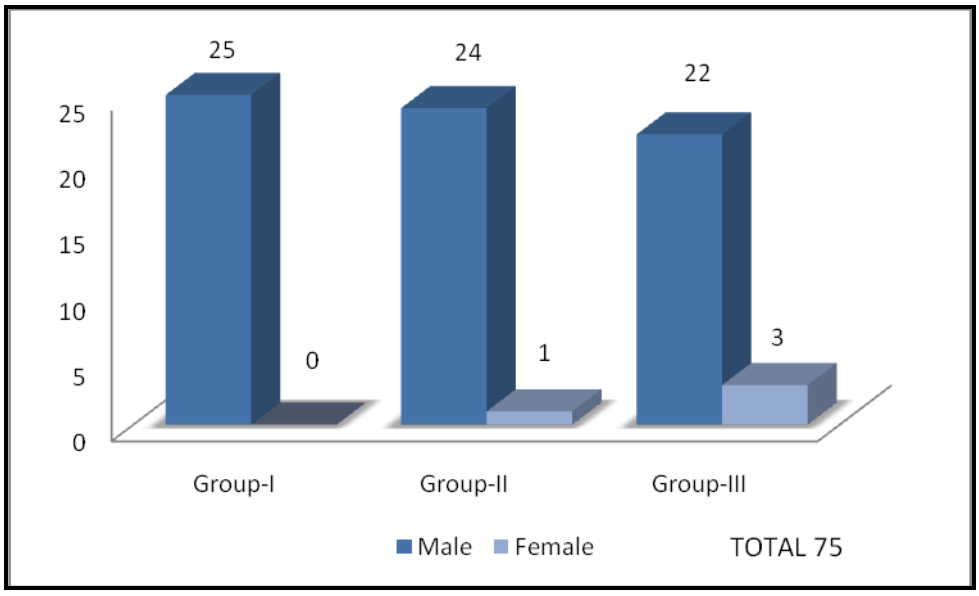
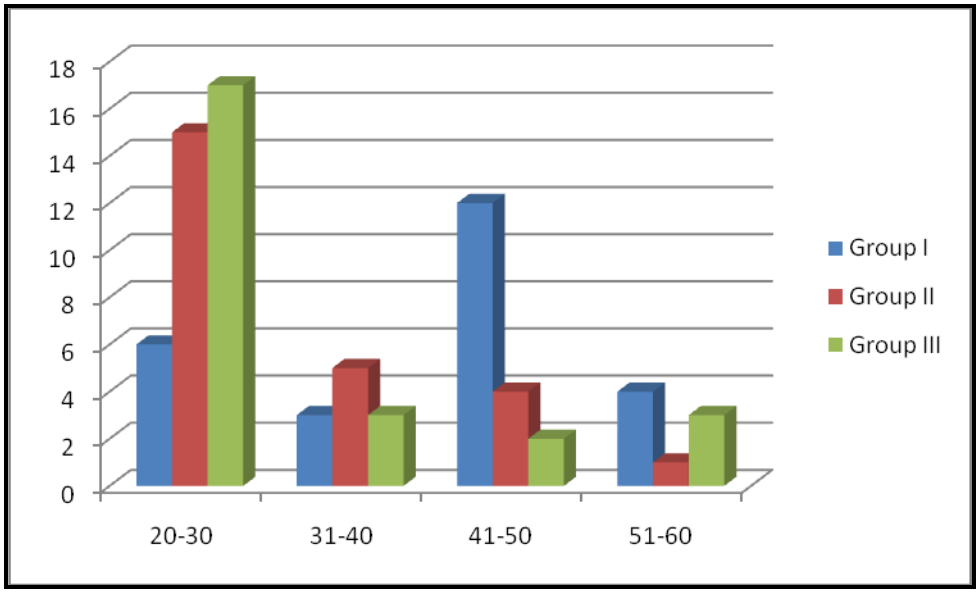
GROUP(A)	GROUP(B)	Mean difference (A) - (B)	Test of significance
GROUP-I	GROUP-II	0.056320	0.0001 (significant)
	GROUP-III	0.030320	0.003 (significant)
GROUP-II	GROUP-I	0.056320	0.0001 (significant)
	GROUP-III	0.086640	0.0001 (significant)
GROUP-III	GROUP-I	0.030320	0.003 (significant)
	GROUP-II	0.086640	0.0001 (significant)

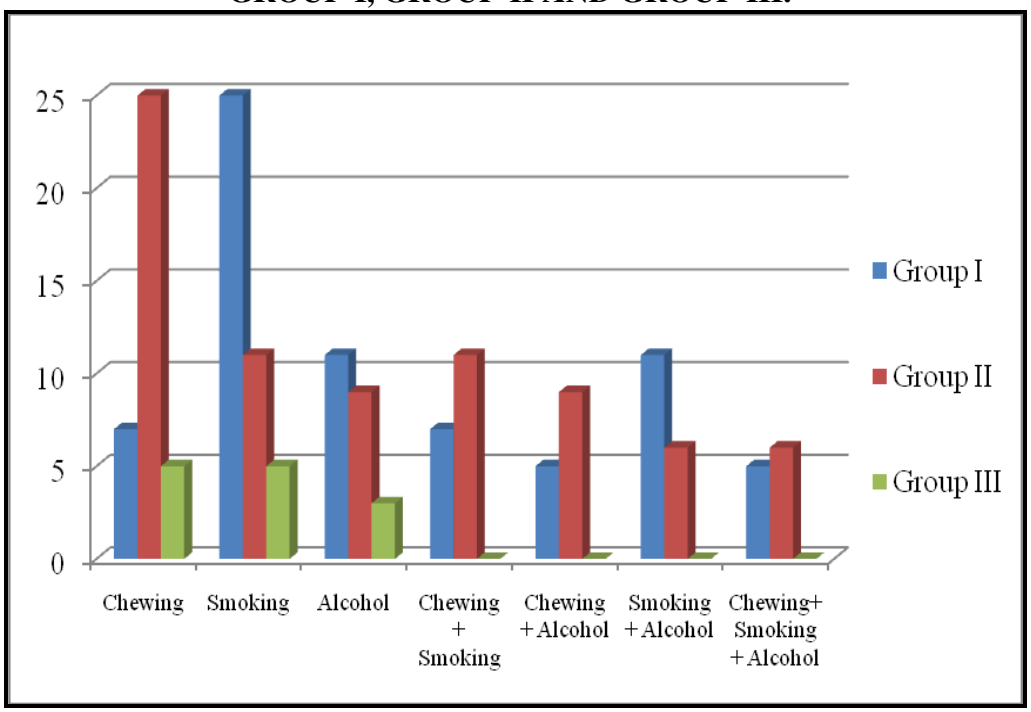
GRAPH-1: DISTRIBUTION OF SUBJECTS IN SEX WISE .



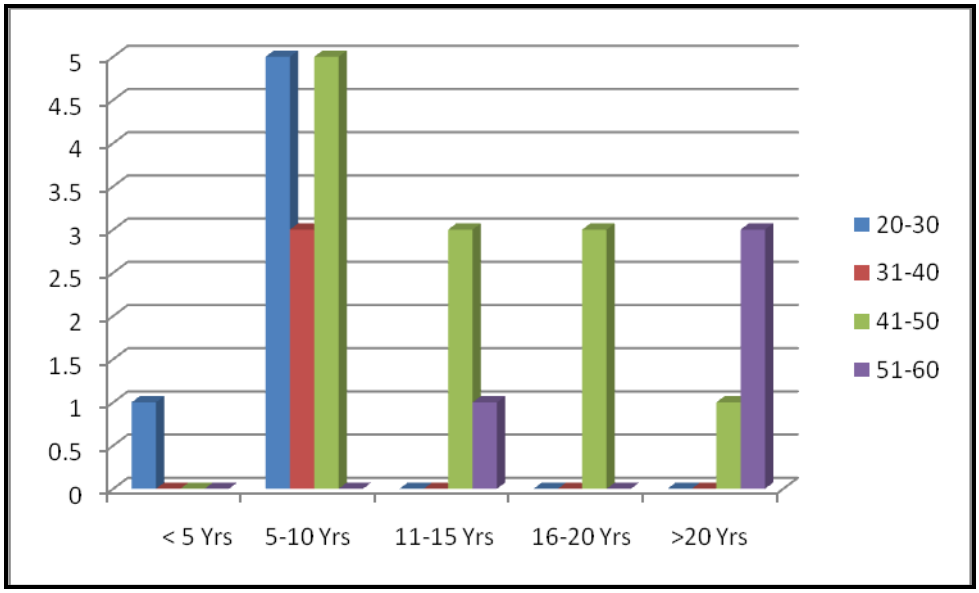
GRAPH – 2: DISTRIBUTION OF SUBJECTS IN AGE WISE



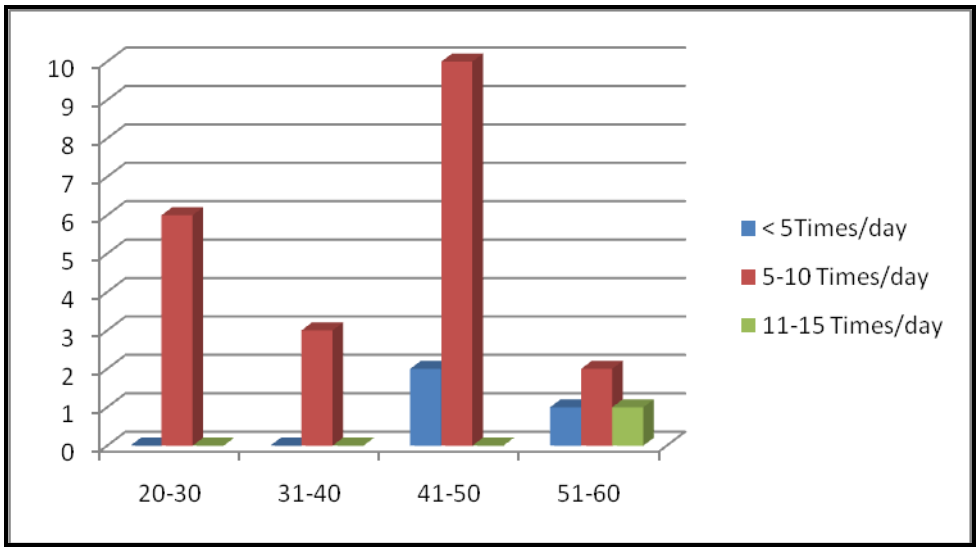
GRAPH – 3: DISTRIBUTION OF SUBJECTS BASED ON HABITS IN GROUP-I, GROUP-II AND GROUP-III.



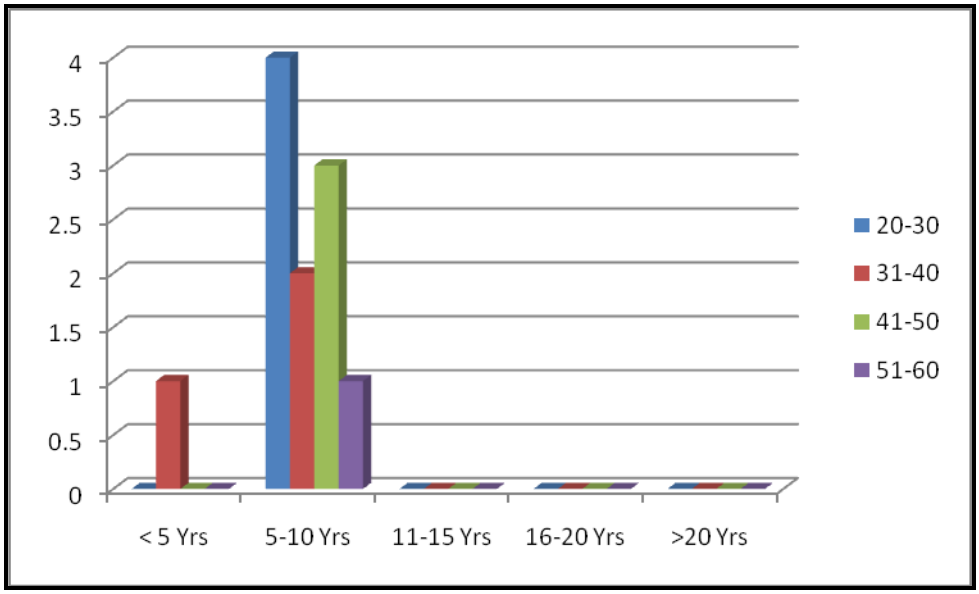
GRAPH – 4: DISTRIBUTION OF SUBJECTS IN AGE WISE BASED ON DURATION OF SMOKING IN GROUP-I



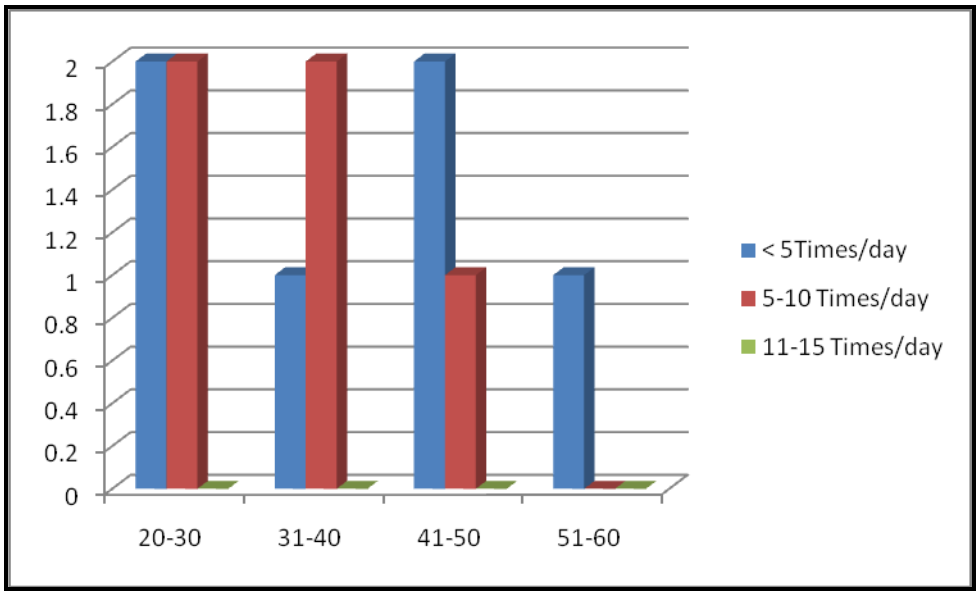
GRAPH – 5 : DISTRIBUTION OF SUBJECTS IN AGE WISE BASED ON FREQUENCY OF SMOKING IN GROUP-I



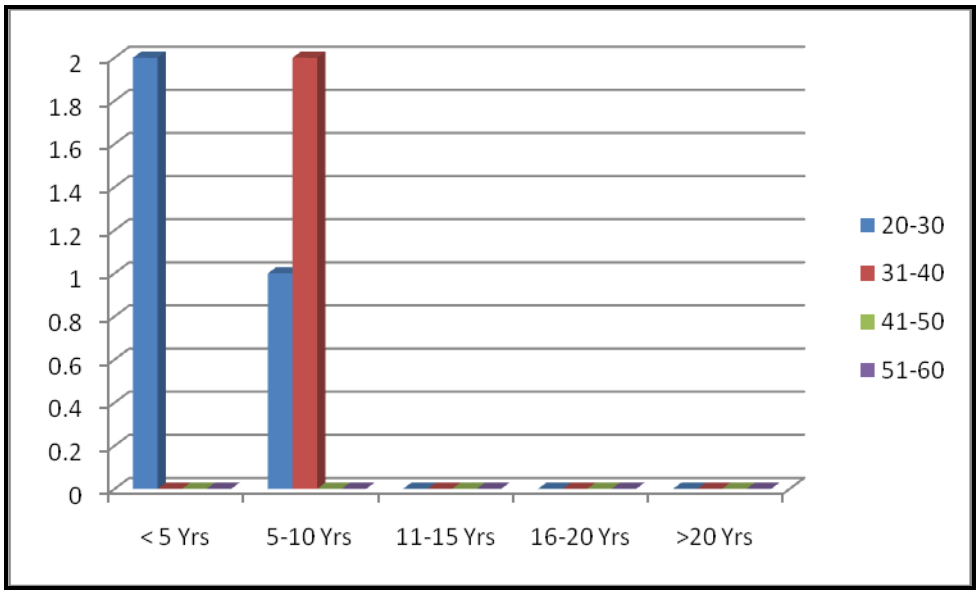
GRAPH – 6 : DISTRIBUTION OF SUBJECTS IN AGE WISE BASED ON DURATION OF SMOKING IN GROUP-II



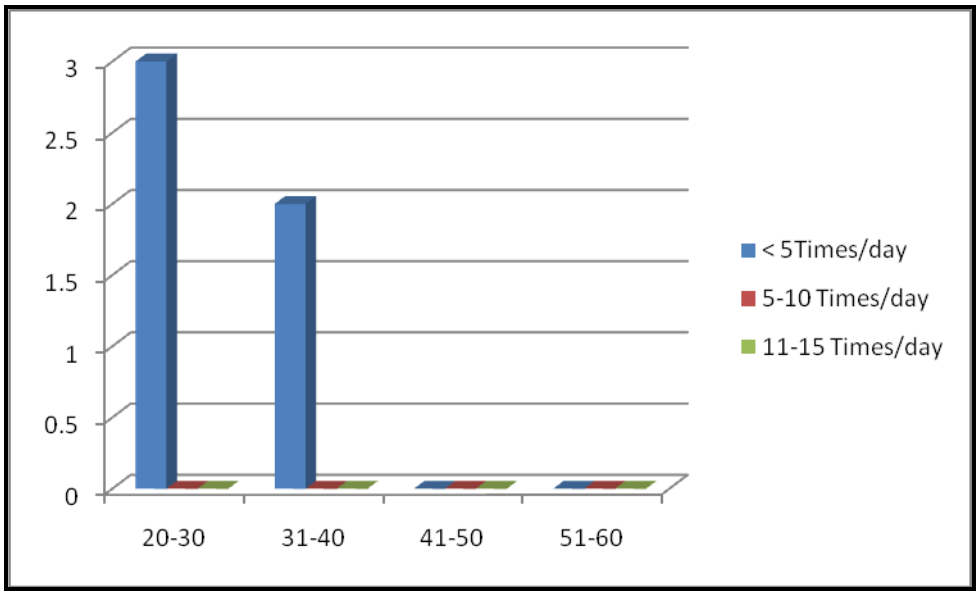
GRAPH – 7: DISTRIBUTION OF SUBJECTS IN AGE WISE BASED ON FREQUENCY OF SMOKING IN GROUP-II



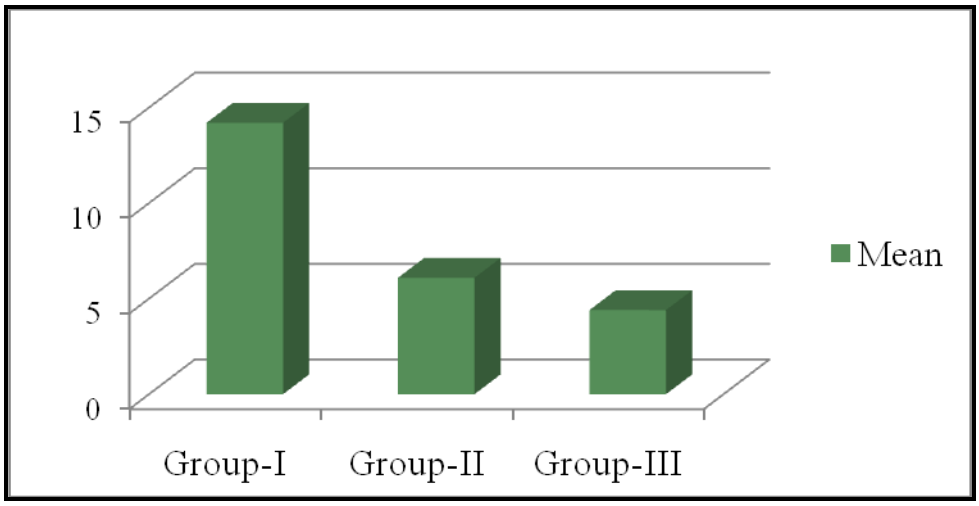
GRAPH – 8: DISTRIBUTION OF SUBJECTS IN AGE WISE BASED ON DURATION OF SMOKING IN GROUP-III



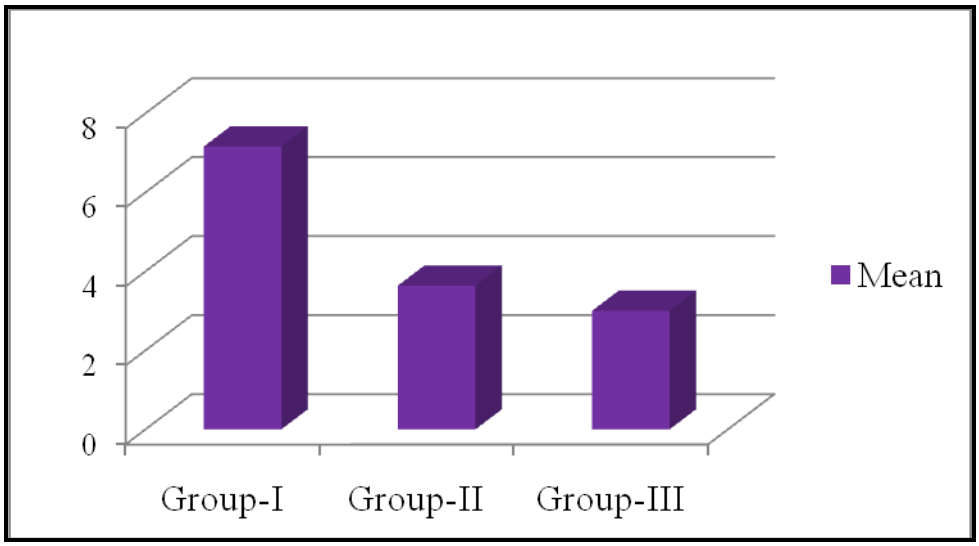
GRAPH – 9: DISTRIBUTION OF SUBJECTS IN AGE WISE BASED ON FREQUENCY OF SMOKING IN GROUP III



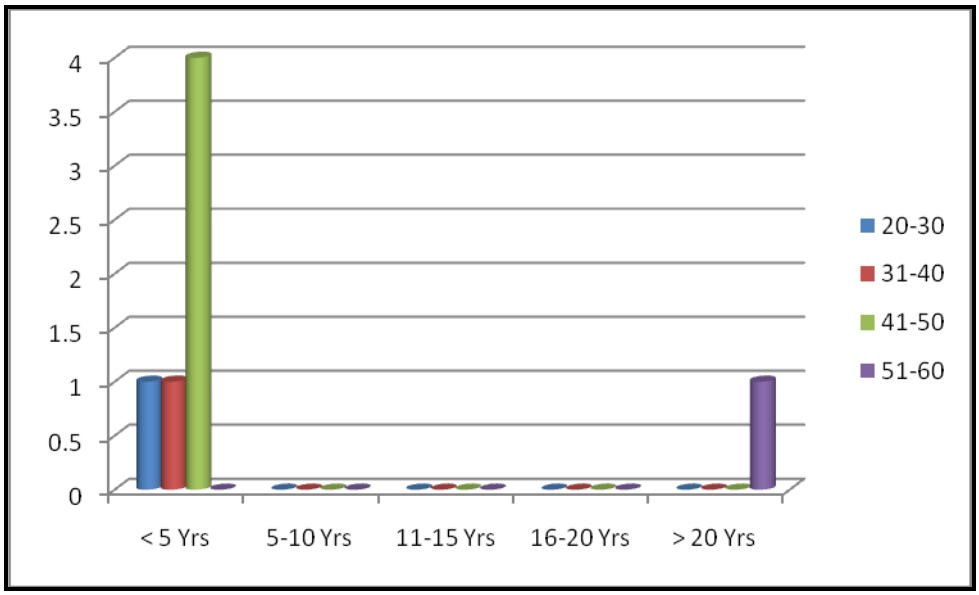
GRAPH 10 . a .DISTRIBUTION OF SMOKING DURATION IN GROUP-I,GROUP-II AND GROUP-III



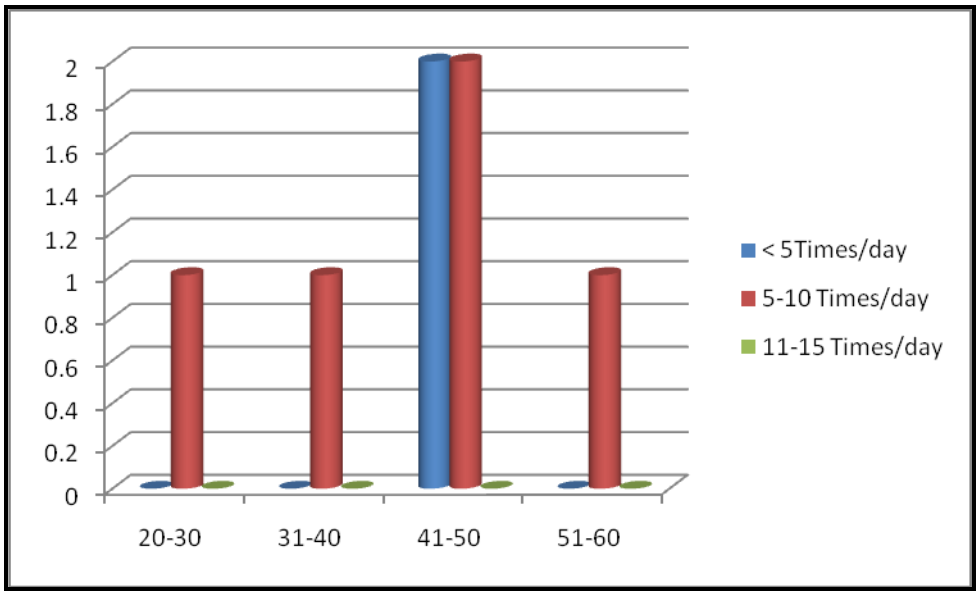
GRAPH 10 . b .DISTRIBUTION OF SMOKING FREQUENCY IN GROUP-I,GROUP-II AND GROUP-III



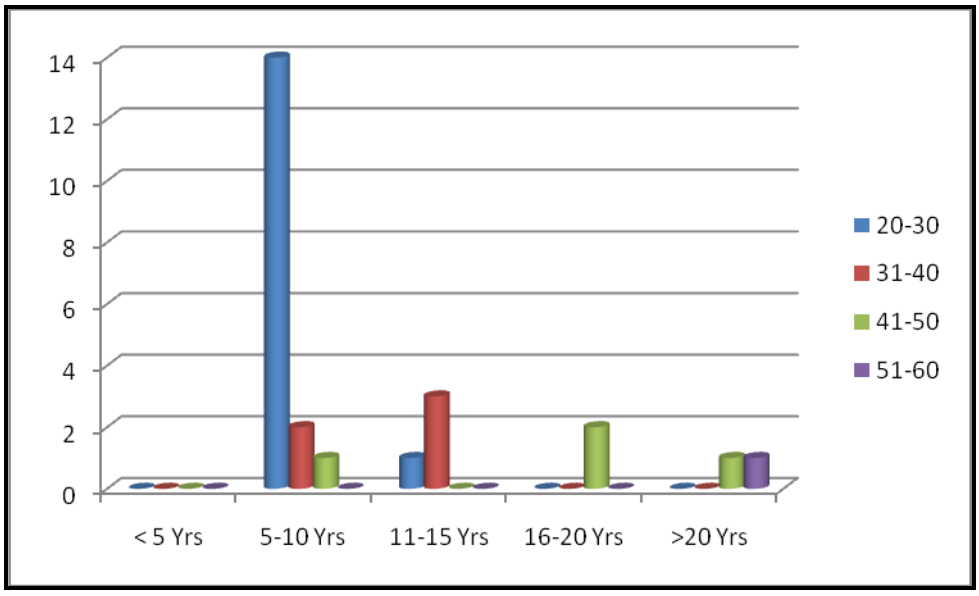
GRAPH – 11: DISTRIBUTION OF SUBJECTS IN AGE WISE BASED ON DURATION OF CHEWING IN GROUP-I



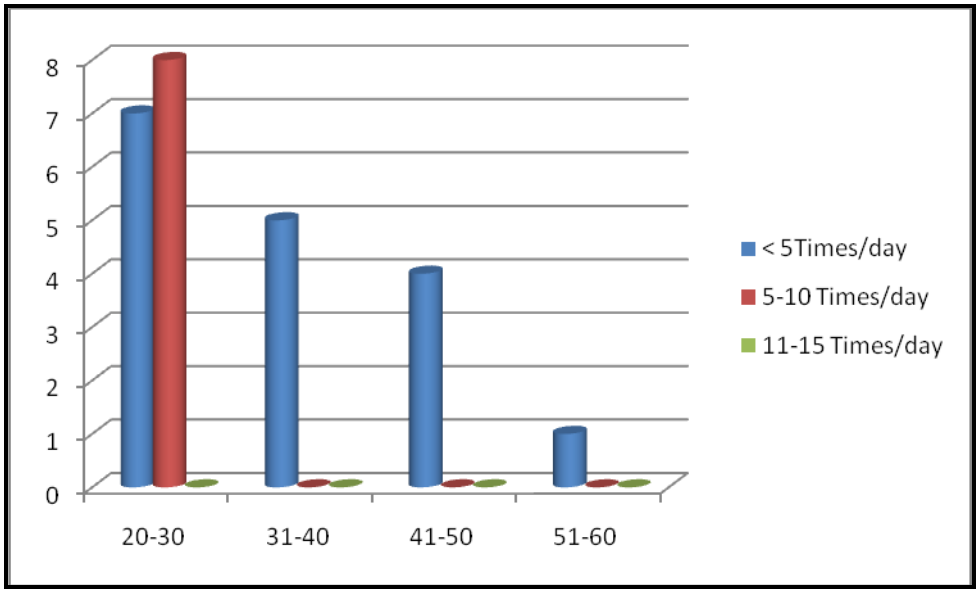
GRAPH – 12: DISTRIBUTION OF SUBJECTS IN AGE WISE BASED ON FREQUENCY OF CHEWING IN GROUP-I



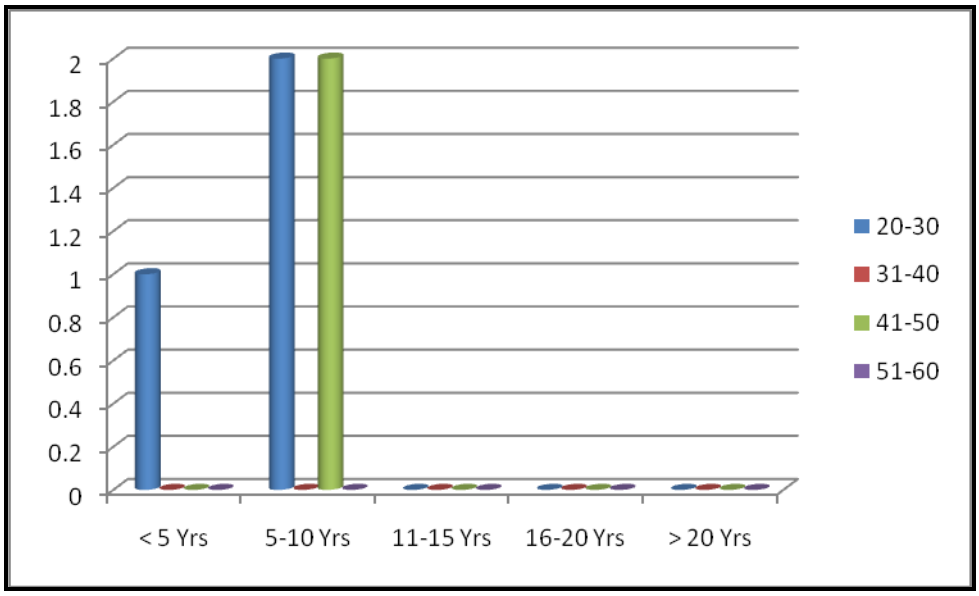
GRAPH – 13. DISTRIBUTION OF SUBJECTS IN AGE WISE BASED ON DURATION OF CHEWING IN GROUP-II



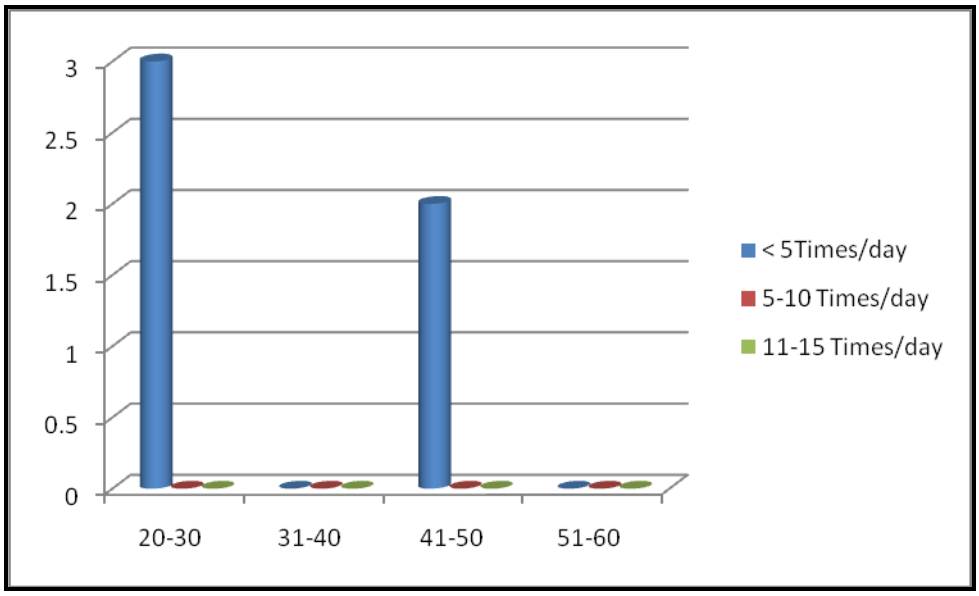
GRAPH – 14. DISTRIBUTION OF SUBJECTS IN AGE WISE BASED ON FREQUENCY OF CHEWING IN GROUP-II



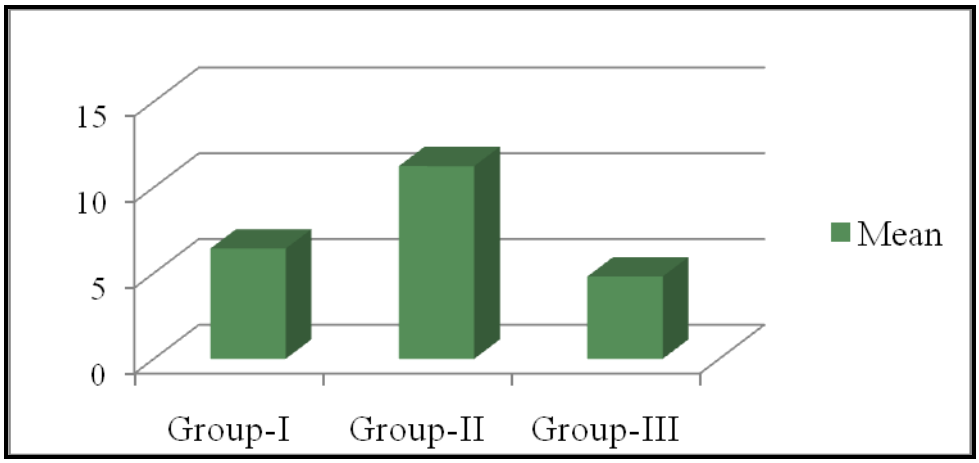
GRAPH – 15. DISTRIBUTION OF SUBJECTS IN AGE WISE BASED ON DURATION OF CHEWING IN GROUP-III



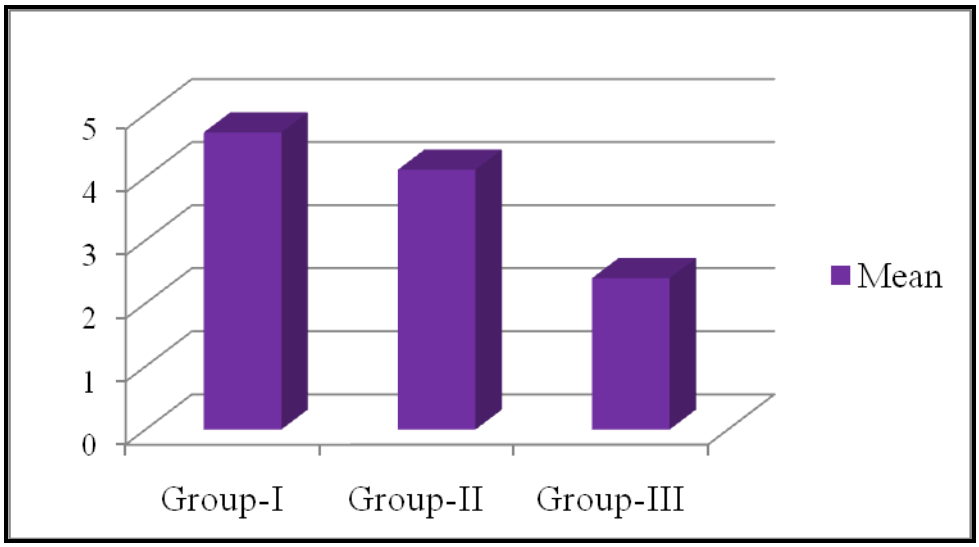
GRAPH – 16. DISTRIBUTION OF SUBJECTS IN AGE WISE BASED ON FREQUENCY OF CHEWING IN GROUP-III



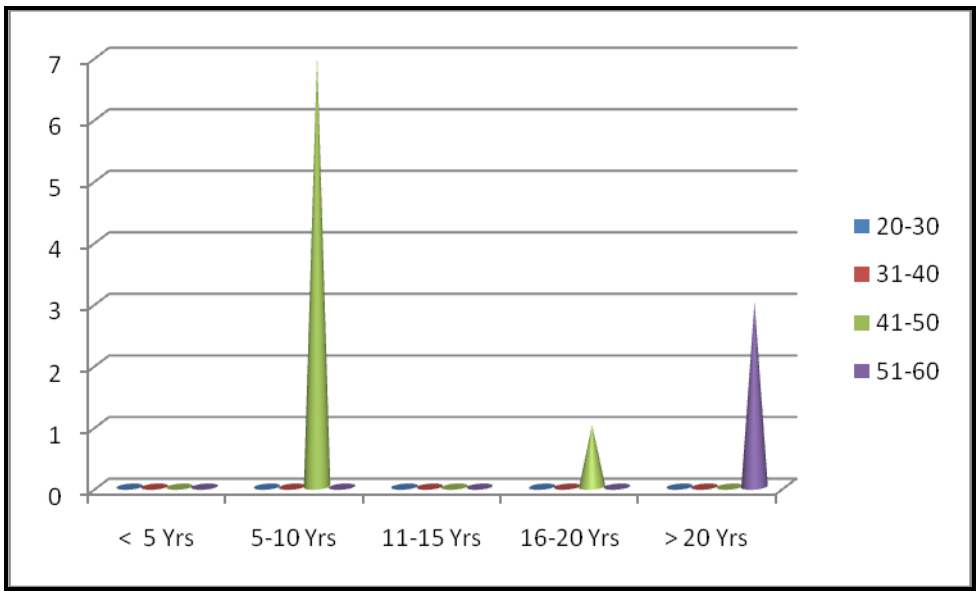
GRAPH 17.a .DISTRIBUTION OF CHEWING DURATION IN GROUP-I,GROUP-II AND GROUP-III



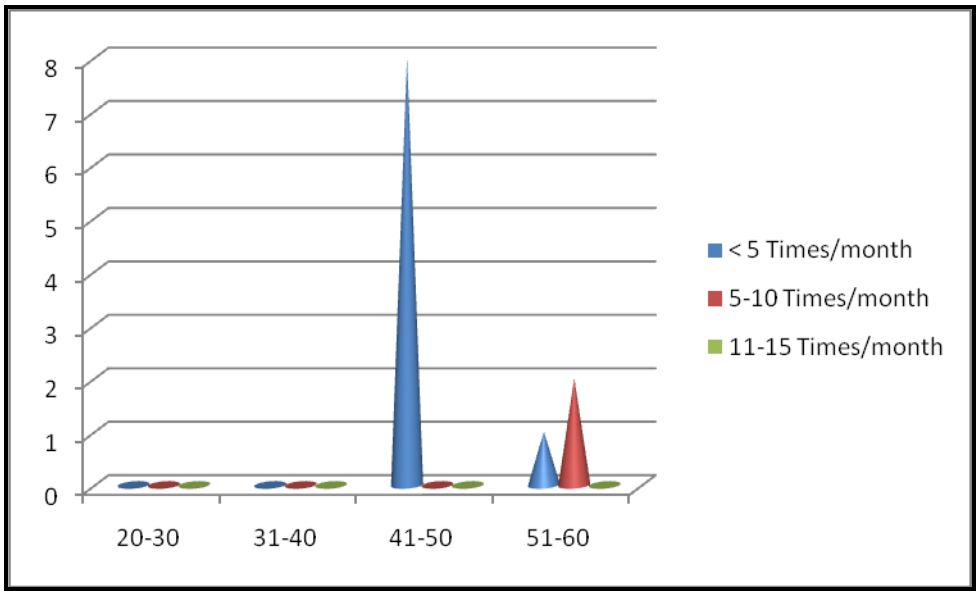
GRAPH 17.b.DISTRIBUTION OF CHEWING FREQUENCY IN GROUP-I,GROUP-II AND GROUP-III



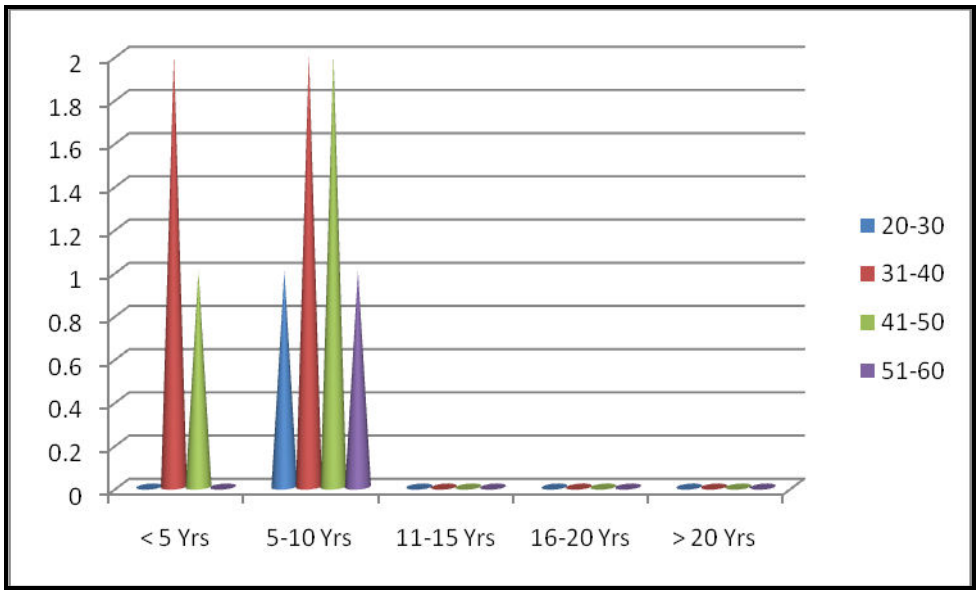
GRAPH – 18. DISTRIBUTION OF SUBJECTS IN AGE WISE BASED ON DURATION OF ALCOHOL CONSUMPTION IN GROUP-I



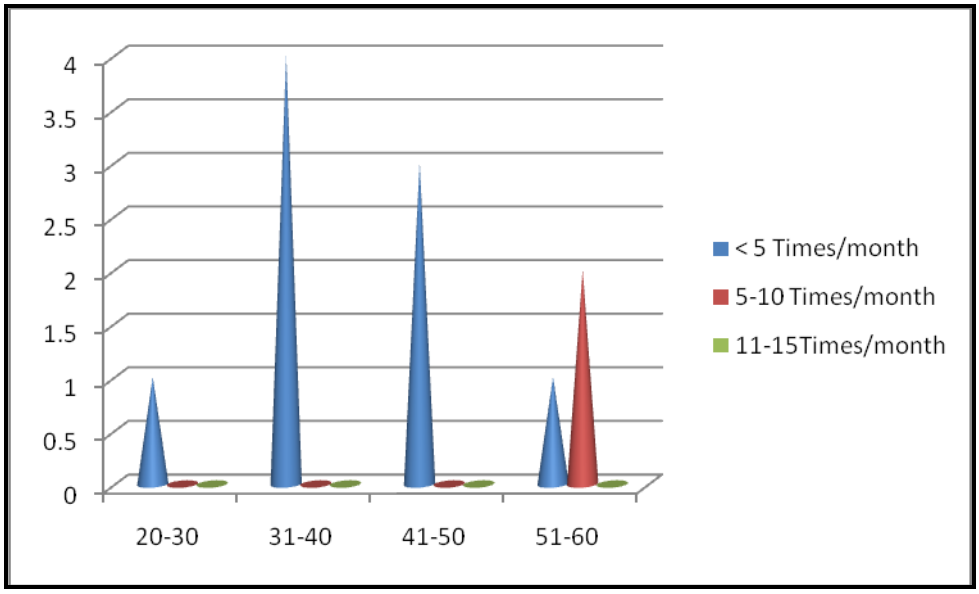
GRAPH –19. DISTRIBUTION OF SUBJECTS IN AGE WISE BASED ON FREQUENCY OF ALCOHOL CONSUMPTION IN GROUP-I



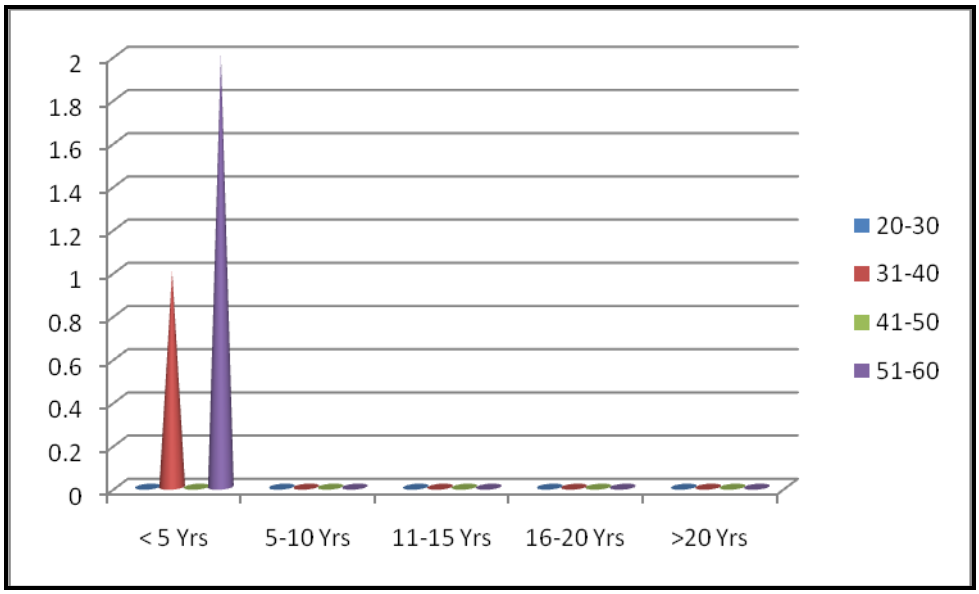
GRAPH – 20. DISTRIBUTION OF SUBJECTS IN AGE WISE BASED ON DURATION OF ALCOHOL CONSUMPTION IN GROUP-II



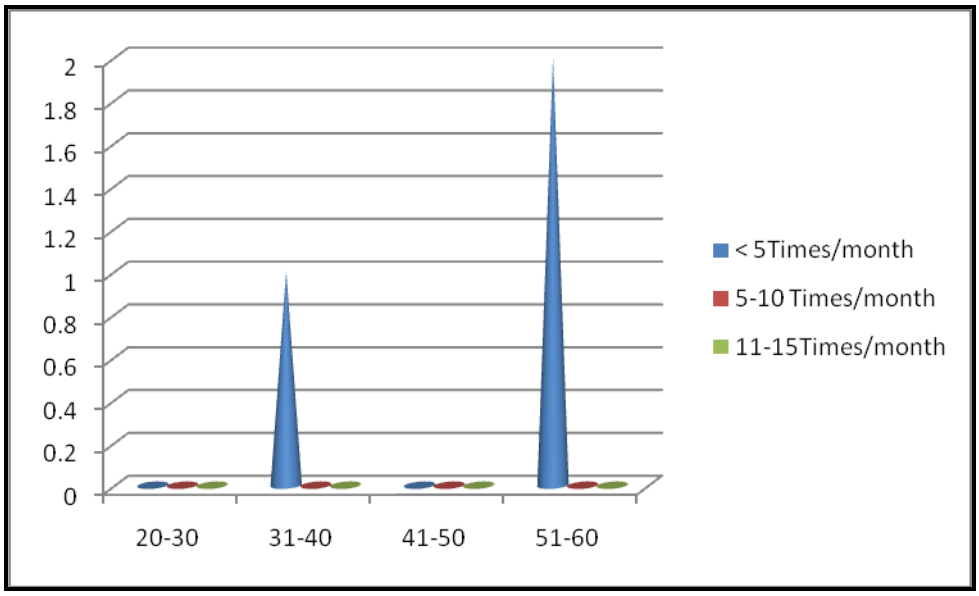
GRAPH – 21. DISTRIBUTION OF SUBJECTS IN AGE WISE BASED ON FREQUENCY OF ALCOHOL CONSUMPTION IN GROUP-II



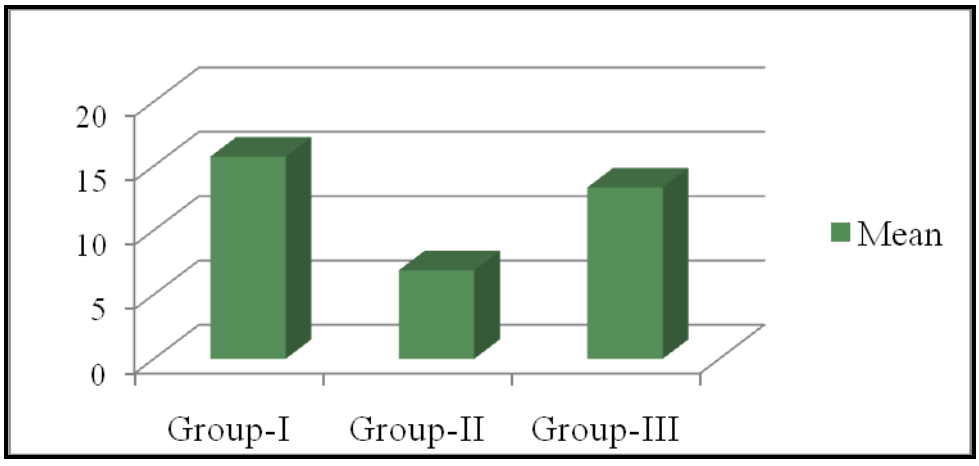
GRAPH – 22. DISTRIBUTION OF SUBJECTS IN AGE WISE BASED ON DURATION OF ALCOHOL CONSUMPTION IN GROUP-III



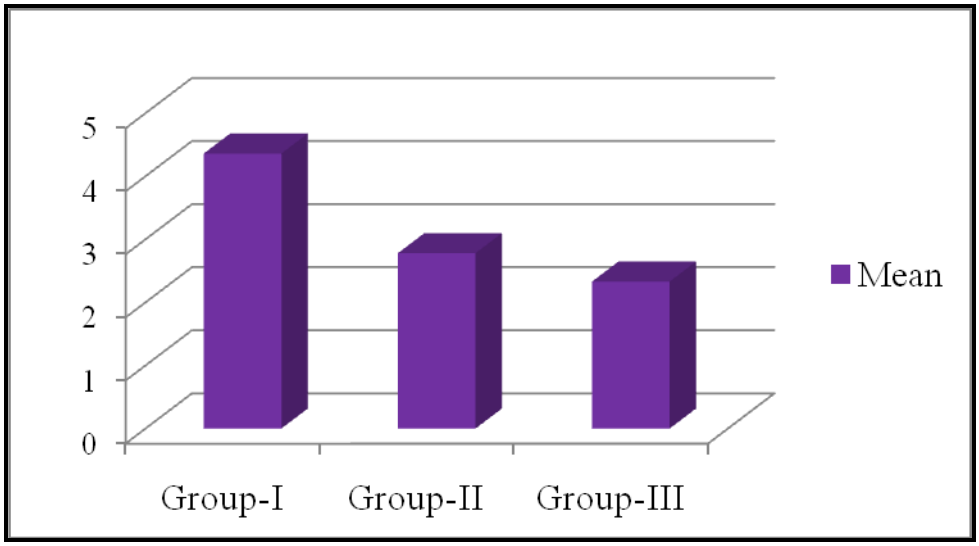
GRAPH – 23. DISTRIBUTION OF SUBJECTS IN AGE WISE BASED ON FREQUENCY OF ALCOHOL CONSUMPTION IN GROUP-III



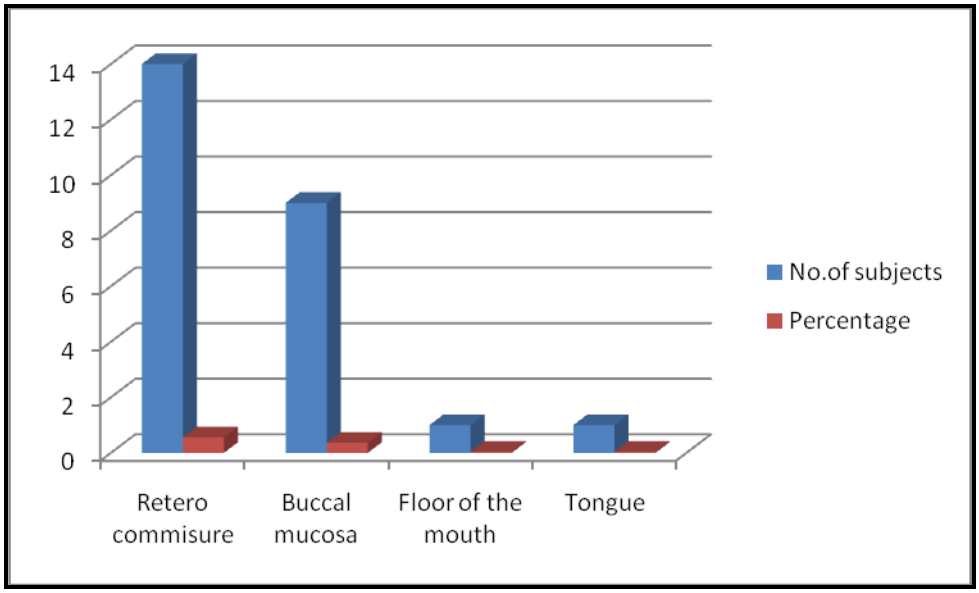
GRAPH 24.a.DISTRIBUTION OF ALCOHOL CONSUMPTION DURATION IN GROUP-I,GROUP-II AND GROUP-III



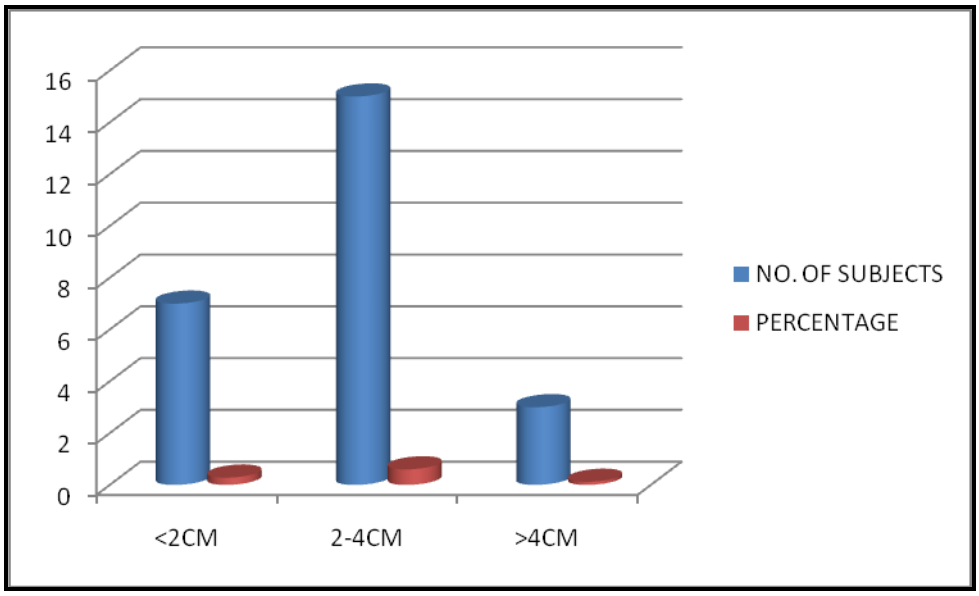
GRAPH 24.b.DISTRIBUTION OF ALCOHOL CONSUMPTION FREQUENCY IN GROUP-I,GROUP-II AND GROUP-III



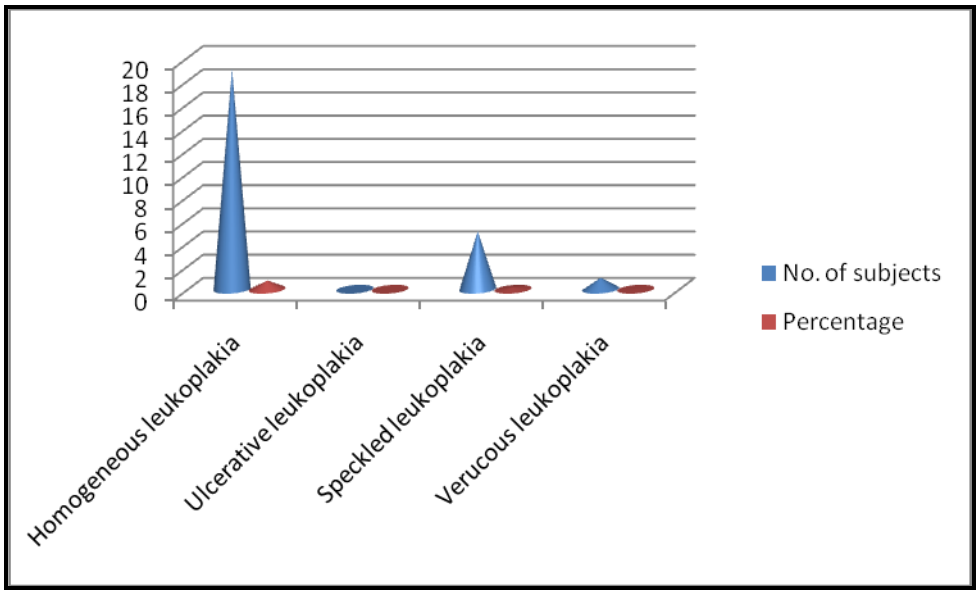
GRAPH 25 .DISTRIBUTION OF SUBJECTS IN GROUP-I ACCORDING TO SITE OF LEUKOPLAKIA



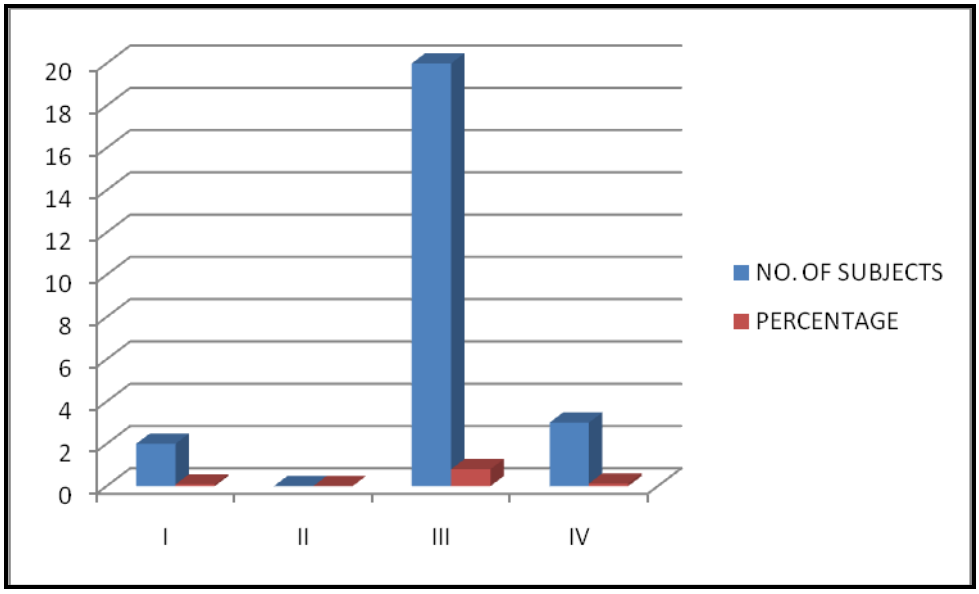
GRAPH -26.DISTRIBUTION OF SUBJECTS IN GROUP-I ACCORDING TO SIZE OF LEUKOPLAKIA



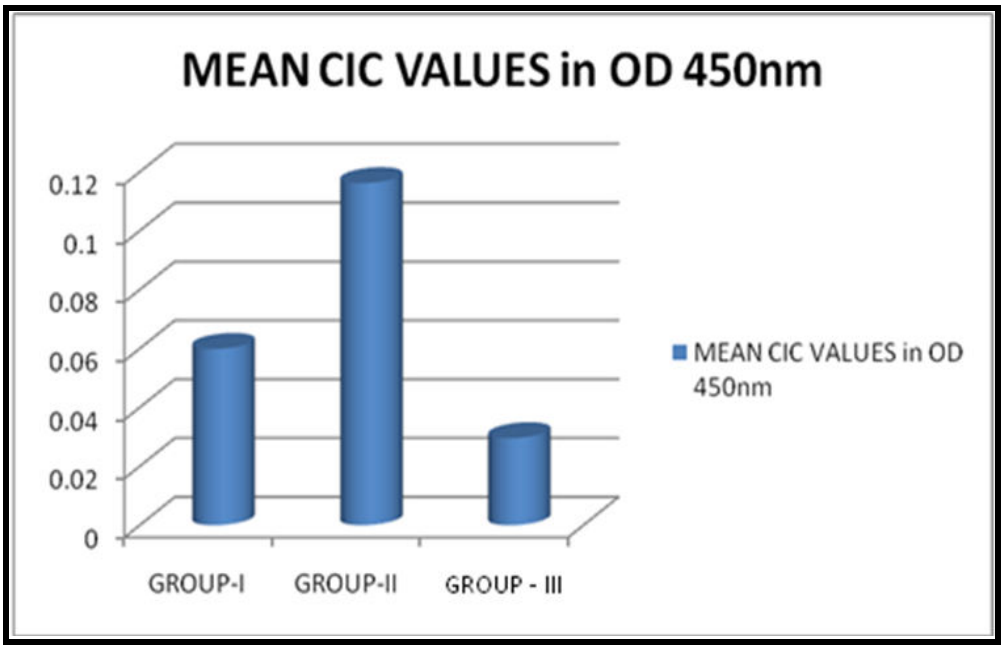
GRAPH -27.DISTRIBUTION OF SUBJECTS IN GROUP-I ACCORDING TO TYPE OF LEUKOPLAKIA



GRAPH -28 DISTRIBUTION OF SUBJECTS IN GROUP II ACCORDING TOCLINICAL GRADE



GRAPH -29.MEAN CIC VALUES IN RELATION TO GROUP-I,GROUP-II AND GROUP-III



In India, oral cancer is prevalent in most areas where tobacco related practices are observed. For development of oral cancer, tobacco is the single greatest risk factor. When this is combined with arecanut the risk increases many fold. Alcohol, viruses, genetic mechanisms, candida, chronic irritation and diet deficiency states are also implicated in the etiology.[73]

Cancer (medical term: malignant neoplasm) is a class of diseases in which a group of cells display *uncontrolled growth* (division beyond the normal limits), *invasion* (intrusion on and destruction of adjacent tissues), and sometimes *metastasis* (spread to other locations in the body via lymph or blood). These three malignant properties of cancers differentiate them from benign tumors, which are self-limited, and do not invade or metastasize**.** [68]

Head and neck malignancies rank among top three malignancies in our country for both males and females. The wide spread use of tobacco in various forms due to our cultural habits coupled with lack of awareness of it carcinogenic effects are responsible for their prevalence. Ignorance of early symptoms together with lack of proper diagnostic and treatment facilities at the gross root level lead to presentation of patients to cancer hospitals in advanced disease status in our country.[21]

Oral cancer is almost always preceded by benign lesions or conditions for varying lengths of time. Interestingly the benign lesions and

conditions also share the same risk factors as the cancer, particularly the tobacco and the areca nut. Many of these have the potential to become cancer and are termed as precancerous lesions and conditions. [21]

Significant reduction in mortality can be achieved by advances in early diagnosis and implementation of multidisciplinary treatment programmes leading to improvement of survivorship and better quality of life.

Among the oral tumors 90% are oral squamous cell carcinoma which arise from the mucosal lining. Inspite of significant advances in surgery, radiotherapy and chemotherapy the 5 year survival rate has remained at about 52% for the past few decades.

The development of oral cancer is a multistep process arising from pre-existing potentially malignant lesions. Leukoplakia is the most common precancer representing 85% of such lesion.[8]

The incidence of Oral sub mucous fibrosis (OSMF) is increasing like an epidemic, targeting the younger generation. The etiology for OSMF is still obscure and a varied number of factors have been proposed. Of these, areca nut use is the most important and persistent finding in history taking. The incidence of malignant changes in patients with Oral sub mucous fibrosis ranges from 3 to 6%.[61]

Intensive studies have documented the role of immune complexes as modulators of both cellular and humoral immune response. The occurrence

of circulating immune complexes (CIC) as a marker for tumor burden and prognosis in the sera of patients with oral precancer and cancer is now well established. Recent advances in the fields of CIC, tumor progression, drug resistance, tumor cell heterogeneity and metastasis have resulted in a renewed interest in the development of non- specific immunotherapeutic modalities.[75,88]

Two types of antigen antibody complex formation can be investigated in the patients with malignant diseases. In the first type anti-tumor antibodies interact with cell-surface associated tumor antigens. Immune complexes formed on the tumor cell surfaces may undergo endocytosis or be released in to the cell environment. In the second type immune complexes are formed when the tumor-associated antigens, shed from tumor cells and circulating in body fluids ,interact with antitumor antibodies. Modified Raji cell technique can be used to estimate the levels of Circulating Immune Complexes.[89]

Under normal conditions it is cleared by phagocytosis. If CIC escape phagocytic clearance they may be deposited in endothelial vasculature and cause tissue damage.

Presuming the involved antigen to be of malignancy specific, serum CIC levels in cancer patients have been used for early diagnosis, metastatic spread, tumor burden, degree of aggressiveness therapeutic response as well as prognosis. [79]

The immunological abnormalities in patients with cancer in the head and neck appear to be more profound than those associated with cancers of the bronchus, breast, cervix, colon or bladder. [41]

The range of tumor markers in human cancer include oncofoetal proteins, enzymes, hormones, polyamines, tumour associated antigens, lipids, Viral markers, circulating immune complexes,immunoglobulins and glycoproteins. Studies have shown increased levels of CIC in oral premalignant and malignant lesions and conditions.

The CIC play a specific role as initiators of mechanism of tissue injury in many infections, autoimmune disorders, and neoplastic diseases. A strong correlation exists between CIC level and progression to cancer.[54]

The overall consensus is that only a small percentage of the detected CIC in vivo represent tumor associated antigens complexed with antibodies. The bulk of CIC most likely represent auto antibodies or the reaction to denatured self proteins, microbes, normal lymphocyte, antigens and nuclear antigens. [76]

Elevated levels of CIC have been found in variety of diseases including neoplasia. The suggestion that CIC might compromise in the host –tumour relationship on the immunological front, has led to extensive studies aiming to unravelling the correlation between CIC levels in serum and clinical course of neoplastic diseases.[3,45]

Antigenic make up of CIC in cancer patients reflects the host's immune response to a variety of often overlapping antigenic stimuli and hence paves way for further studies.[77]

In the present study the levels of CIC show a gradual increase in the precancer group and the cancer group. It is characterized by a marked increase in levels which is statistically significant. From these results it can be hypothesized that CIC represent the host's physiological and immunological defense response in eliciting specific antibodies upon exposure to most antigenic substances.

It also leads to suppression of cell mediated immunity and modulates the humoral response.[77,91]

Immunological and biochemical alterations in the sera of such patients can help not only in early diagnosis, appropriate treatment but also as indicators of prognosis, as the disease progresses.[78]

The present study was done to validate Circulating immune complexes as biological marker for Oral Leukoplakia and Oral submucous fibrosis. In the study the levels of Circulating immune complexes in subjects with untreated Oral Leukoplakia and untreated Oral submucous fibrosis were determined

The CIC levels were compared with Oral Leukoplakia group and Oral submucous fibrosis group and with the control group Finally the relation between circulating immune complexes with oral leukoplakia and Oral submucous fibrosis were determined.

This is a Randomized hospital based case control study conducted between April 2009 to May 2010.

Study population:

Study population includes all subjects reporting to Ragas Dental college and Hospital,Out patient Department,uthandi seeking dental advice and who are from a wide variety of socioeconomic background. The age group selected was between 20-60 years.

Study sample:

A total number of 75 patients were involved in the study.

a) Patients with Oral leukoplakia-Group-I : 25
b) Patients with Oral submucous fibrosis –Group-II: 25
c) Normal controls-Group-III : 25

Permission from the ethical committee of Ragas Dental College and Hospital, Chennai was obtained before starting the study for interpretation and examining subjects,for drawing 5ml of blood .

Also an informed consent was obtained from the subjects forming the study sample,both in English and Tamil to participate in the study and to undergo blood investigation in the course of study.

Data analysis:

The CIC value of each sample is expressed in Optical Density of 450 nm and were tabulated.

All the datas were entered in Microsoft excel sheets. Statistical analysis was done using SPSS software SYSTAT version 7.0.

Mean values were compared by using one-way ANOVA followed by multiple range tests by Tukey-HSD procedure.

The test of significance with p value less than 0.05 at 95% confidence interval is taken to correlate the variables to determine the significance.

The results are compared to similar study performed by **Cynthia Jane in 2007 [15].**with reference to dependent variable Circulating immune complex in Oral leukoplakia,Oral submucous fibrosis and control groups.

STUDY ANALYSIS:

AGE:

The subjects were divided in to four age groups which are as follows:20-30yrs,31-40yrs,41-50yrs,51-60yrs to enhance the statistical analysis. It was found that the age wise distribution of subjects were found to be statistically not significant, which means that both the experimental and control groups were similar with respect to age distribution.

The Oral leukoplakia group had maximum number of cases in the age group of 41-50years,with 12 subjects (44%),that is in the fifth decade of life,and is consistent with the studies done by **R.Rajenderan et al 2006. [68]**

In Oral sub mucous fibrosis group the prevalence of age group is much more younger than oral leukoplakia age group. That is 15 (60%)

subjects lie between the age group of 20-30 years which is consistent with the study done by **Babu S, in 1996 (58%).** [4]

In a study done by **Gupta PC in 1998** [32] arecanut usage was concentrated in the lower age group less than 35 years. They found upto 85% cases were below 35years, which is also in consistent with our study.

SEX:

In the present study oral leukoplakia was completely seen in males only, with 25 subjects(100%) which is exactly matching with the study done by **Hogewind WFC and Van Der I in 1989 (100%).**[34]

In the present study 24 males (96%) and 1 female (4%) were clinically diagnosed having oral submucous fibrosis,which is in accordance with the study of **Dayal.P.K.Punnnya V et al in 1998 (89% -M; 11%-F).**[17]

In the present study since only one female subject is included in experimental group, combining the oral leukoplakia and oral submucous fibrosis tabulation correlating the sex and the age group has not been given. The only female subject in the OSMF group lies in the age group of 20-30yrs.

HABITS :

In the present study in Oral leukoplakia group 25 subjects (100%) were found with the habit of smoking tobacco. Other habits such as chewing arecanut,alcohol consumption also seen in this group. However the predominant form of the habit is smoking tobacco which is in consistent

with the study of **Holmstrup P et al in 2006** [35] in which they found that 73% of leukoplakia being associated with tobacco habit, the value in the present study is slightly higher than what they reported**.**

The study done done by **Mehta et al in 1972** [47] showed the incidence of Oral leukoplakia is more with smoking tobacco with 4.2 per 1,00,000 when compared with betel quid which is 1.3 per 1,00,000 and those who do not use tobacco ,which is 0.6 per 1,00,000.

In the Oral sub mucous fibrosis group areca nut chewing habit predominated 25 subjects (100%) and can be predicted as the only etiological factors associated with OSMF. The subjects also had habits of smoking and alcohol consumption but to varying degree. This is in accordance with the study done by **Maher et al in 1994** [42] where they found 98% of subjects were with the habit of chewing areca nut who suffered from OSMF.

By comparing Oral leukoplakic group, Oral submucous fibrosis group and the control group with the habit as smoking, chewing and alcohol consumption by using one way ANOVA it was found that there exists significant correlation among the groups in relation to habits with p value 0.035.

Distribution of subjects with habit of smoking in oral leukoplakia,oral submucous fibrosis and control groups revealed that there exists significant correlation among the groups with respect to smoking frequency with p value 0.0002 and smoking duration with p value 0.003.

Distribution of subjects with habit of chewing in oral leukoplakia, Oral sub mucous fibrosis and control groups revealed that there exists significant correlation among the groups with respect to chewing frequency with p value 0.030,and there is no significant correlation with respect to chewing duration with the p value 0.085 which is in accordance with the study done by **Shah N and Sharma PP in 1998** [83] where they found frequency of chewing is correlated to the development of OSMF than duration of chewing.

Distribution of subjects with habit of alcoholism in oral leukoplakia, Oral submucous fibrosis and control groups revealed that there exists no significant correlation among the groups with respect to alcohol consumption frequency with p value 0.235 and smoking duration with p value 0.098,which can be attributed to the fact that the habit of alcoholism is much lesser in the three groups, with Oral leukoplakia-11 subjects (44%),Oral submucous fibrosis -09 subjects (36%) and control group 03 subjects (12%) and also requires larger sample size to find the correlation between the groups.

SITE:

In subjects with predominantly smoked form of tobacco habit,14 subjects(56%) had lesions in the reterocommisural area,followed by 9 subjects (36%) had lesions in buccal mucosa,1subject (4%) had lesion in the floor of the mouth and I subject had lesion in the tongue(4%) which is consistent with the study conducted by **Reichart PA and Kohn H in**

1996 [70] ,in which they found the reterocommisure was the most commonly affected site upto 43.35% followed by 36.7% in buccal mucosa.

In a 10 year followup study conducted in Mumbai **Napier SS and Speight PM.in 2008** [49] 80% lesions were found in reterocommisure and buccal mucosa and 5% found in tongue . The present study is consistent with this report also.

SIZE:

In the present study the size of the oral leukoplakia is more with 2-4 cm with distribution of 15 subjects(60%) ,followed by less than 2cm in 7 subjects(28%) and greater than 4 cm in 3 subjects (12%),which is not in consistent with the study done by **Pindborg J.J et al in 1968** [57]**.**

CLINICAL TYPE:

In the present study it was found that most of the cases were of homogeneous leukoplakia with 19 subjects (76%),followed by speckled leukoplakia with 5 subjects (20%) and Verrucous leukoplakia in 1 subject (4%) . No ulcereative leukoplakia was seen in our study. This study is in accordance with the study conducted by **Reichart PA and Kohn H in 1996** [70] where homogeneous type to be more (84.2%) than nonhomogeneous type (15.8%).

Non homogeneous leukoplakia accounted for the highest frequency of malignant transformation of 20%, whereas 3% of the homogeneous leukoplakia developed into carcinoma [12].

In the present study out of 7 cases 4 homogemous and 3 non homogenous leukoplakia showed high CIC values which is above Optical Density 450 nm -0.08 deviates greatly from the mean value from the Group I leukoplakia which is 0.05988, this may be attributed to the fact that these lesions carries a high relative risk for malignant transformation ,which is in accordance with the study done by **Cynthia Jane in 2007 [15] (p< 0.002).**

CLINICAL GRADING:

In clinical grading of OSMF grade III condition predominated with 20 subjects (80%) which is followed by Grade IV and Grade –I. No subjects were found in Grade II,GradeV and Grade VI.

This Grade III predominance of clinical presentation can be attributable to the fact ,that the presence of blanching and burning sensation, dryness of mouth, vesicles or ulcers in the mouth with restriction of mouth opening and palpable bands all over the mouth with out tongue involvement which causes major morbidity to the patient making them to seek dental advice.

CIC IN ORAL LEUKOPLAKIA AND ORAL SUBMUCOUS FIBROSIS:

The occurrence of CIC is a never in any normal immune response. The half-life of such CIC is transitory in nature. Continued presence of CIC over extended periods however is a cause of consequence of some pathological condition or infection .[15]

Presuming the involved antigen to be of malignancy-specific,serum CIC levels in cancer patients have been used for early diagnosis,metastatic spread,tumour burden,degree of aggressiveness, therapeutic response as well as prognosis.[45]

In our study there is marked elevation of CIC values from Normal control to oral leukoplakia then to OSMF which is in accordance with the study conducted by **Cynthia Jane in 2007 [15] (Mean values in OD 450nm Normal-0.02315,Oral Leukoplakia-0.03817,Oral submucous fibrosis-0.1871).**

In the present study out of 25 subjects 4 homogemous and 3 non homogenous leukoplakia showed high CIC values which is above OD450nm -0.08 deviates greately from the mean value from the Group I leukoplakia which is 0.05988. This may be attributed to the fact that these lesions carries a high relative risk for malignant transformation ,which is in accordance with the study by **Cynthia Jane in 2007 [15] ($p < 0.002$).**

Non homogeneous leukoplakia accounted for the highest frequency of malignant transformation of 20%,whereas 3% of the homogeneous leukoplakia developed carcinoma.[12]

In the same way OSMF subjects with clinical Grade IV showed high levels of CIC of OD 450 >0.2 which can be attributed to malignant transformation which is in accordance with the study by **Cynthia Jane in 2007 [15] ($p < 0.001$).**

Since no histopathological study is done in the present study the confirmed malignant transformation of these lesions could not be provided.

In the present study the mean CIC level in normal control is 0.02956, in oral leukoplakia is 0.05988, OSMF is 0.01162 and. It correlates significantly with p value of 0.0001 which is in close accordance with the mean values given in the study and the significance observed in study conducted by **Cynthia Jane et al in 2007** [15] where **p<0.001 .**

MEAN, STANDARD DEVIATION, TEST OF SIGNIFICANCE OF CIC IN RELATION TO NORMAL CONTROL, ORAL LEUKOPLAKIA, OSMF IN THE PRESENT STUDY.

GROUP	MEAN CIC VALUES in OD 450nm	STANDARD DEVIATION	Minimum CIC value in OD 450 nm	Maximum CIC value in OD 450 nm
NORMAL CONTROL	0.02956	0.008912	0.012	0.045
ORAL LEUKOPLAKIA	0.05988	0.024761	0.014	0.089
ORAL SUBMUCOUS FIBROSIS	0.11620	0.046957	0.048	0.251

P value: 0.0001 (p<0.001) (significant) in comparison with the normal controls.

MEAN,STANDARD DEVIATION,TEST OF SIGNIFICANCE IN RELATION TO NORMAL CONTROL,ORAL LEUKOPLAKIA,OSMF, IN THE STUDY DONE BY CYNTHIA JANE ET AL IN 2007 [15]

GROUP	MEAN CIC VALUES in OD 450nm	STANDARD DEVIATION	Minimum CIC value in OD 450 nm	Maximum CIC value in OD 450 nm
NORMAL CONTROL	0.02315	0.013461	0.010	0.046
ORAL LEUKOPLAKIA	0.03817	0.016808	0.013	0.085
ORAL SUBMUCOUS FIBROSIS	0.1871	0.054718	0.045	0.253

P value :p<0.001 in comparison with the normal controls.

The mean levels of CIC in each category of patients differ significantly from the mean level in the normal subjects. This is denoted by p<0.001 which is significant. Since these complex represents the host defence against tumour antigen by production of antibodies the results obtained were obvious in such a way that the CIC value is getting raised from normal to Premalignancy state.

Rajenderan et al in 1986 [65] has reported a depression in the cell mediated and humoral immunity in premalignant lesions of oral cavity. The results of the present study however clearly show that there is an elevation in the CIC in patients with OSMF whereas minimal changes were observed in Oral leukoplakic groups.

This may be attributed to the fact that OSMF carries a high relative risk for malignant transformation. It is thus clear that the level of CIC may be of help in predicting the malignant transformation of OSMF which is in accordance with the similar study done by **Remani et al in 1988** [72] . It is thus clear that the level of CIC may be of help in predicting the malignant transformation of OSMF.

Although further work on composition of CIC will enhance the usefulness of CIC determination in malignant disease, antigenic makeup of CIC in premalignant lesions and conditions reflects the host's immune response to a variety of often overlapping antigenic stimuli and hence paves way for further studies.[77]

Correlating the association between the CIC , and immunoglobulins as IgG,IgM and IgA, CIC and IgA can be used for as the prognostic indicator for premalignant and malignant lesions as suggested by **Sameena parveen et al in 2010 .**[79]

The test of significance using Tukey HSD method revealed significant correlation among the three groups with respect to the dependent variable CIC. There also existed significance mean difference between the Groups I, II, III.

The p value between Group-I and Group-II is 0.0001 ($p<0.05$)

The p value between Group I and GroupIII is 0.003 ($p<0.05$)

The p value between Group-II and Group-III is 0.0001 ($p<0.05$),

which is in accordance with the study done by **Cynthia jane et al in 2007** [15].

To summarize,

TEST OF SIGNIFICANCE BETWEEN GROUP-I,GROUP-II,GROUP-III IN RELATION TO THE DEPENDENT VARIABLE : CIRCULATING IMMUNE COMPLEX

GROUP(A)	GROUP(B)	Mean difference (A)-(B)	Test of significance
GROUP-I	GROUP-II GROUP-III	0.056320 0.030320	0.0001 (significant) 0.003(significant)
GROUP-II	GROUP-I GROUP-III	0.056320 0.086640	0.0001(significant) 0.0001(significant)
GROUP-III	GROUP-I GROUP-II	0.030320 0.086640	0.003(significant) 0.0001(significant)

The mean difference is significant at the 0.05 level between the three Groups I,II,III with p value < 0.0001 (Significant).

The results obtained in the present study are comparable with the study of **Cynthia Jane et al in 2007** [15] which clearly shows the raise in CIC levels from,

NORMALCONTROLS→ORAL LEUKOPLAKIA→ORAL SUBMUCOUS FIBROSIS.

These efforts may be of value for proactive intervention, especially in high risk groups with potentially malignant lesions and conditions, as the early detection decreases the morbidity and the mortality of the disease.

Head and neck malignancies rank among top three malignancies in our country for both males and females. Ignorance of early symptoms together with lack of proper diagnostic and treatment facilities at the gross root level lead to presentation of patients to cancer hospitals in advanced disease status in our country.[21]

Oral cancer is almost always preceded by benign lesions or conditions for varying lengths of time. Interestingly the benign lesions and conditions also share the same risk factors as the cancer, particularly the tobacco and the areca nut. Many of these have the potential to become cancer and are termed as precancerous lesions and conditions. [21]

Intensive studies have documented the role of immune complexes as modulators of both cellular and humoral immune response. The occurrence of circulating immune complexes (CIC) as a marker for tumor burden and prognosis in the sera of patients with oral precancer and cancer is now well established.[75, 88]

The present case control study is done to estimate the circulating immune complexes in potentially malignant disorders as, Oral leukoplakia and Oral submucous fibrosis with equally matched control groups in terms of descriptive and continuous variables.

Patients were clinically examined and after obtaining consent and relevant demographic data ,diagnosis was made with the respective clinical criteria for Oral leukoplakia and Oral sub mucous fibrosis.

Circulating immune complexes were estimated in the serum of control and the experimental group. Various variables such as age, sex, smoking habit, chewing habit, alcoholism, site, size, type of Oral leukoplakia, grade of Oral submucous fibrosis and CIC level in serum were statistically analysed.

To summarise the datas the following observations were made.

1. It was found that the age wise distribution of subjects were found to be statistically significant with p value -0.001. Oral leukoplakia group had maximum number of cases in the age group of fifth decade of life. In Oral sub mucous fibrosis group the prevalence of age group is much more younger than oral leukoplakia age group.

2. The most prevalent habit among the study subjects was smoking, chewing , alcoholism, followed by, chewing and smoking, smoking and alcohol, chewing and alcohol. Subjects with all the three habits seem to be in the last in the order of prevalency. The distribution of subjects based on the habits was found to be significant with the p value-0.035.

3. Distribution of smoking duration and frequency in Group-I,Group-II and Group-III has got correlation among the three groups which is denoted by the p values, for smoking duration **: 0.003 (significant),**for smoking frequency : **0.0002 (significant)**

4. Distribution of chewing frequency in Group-I,Group-II and Group-III has got correlation among the three Groups which is denoted by the p value for chewing frequency **: 0.030 (significant).**

5. In subjects with predominantly smoked form of tobacco habit had lesions in the reterocommisural area,followed by buccal mucosa, floor of the mouth and tongue.

6. In the present study the size of the oral leukoplakia is more with 2-4 cm.

7. In the present study it was found that most of the cases were of homogeneous leukoplakia, followed by speckled and Verrucous leukoplakia.

8. In clinical grading of OSMF grade III condition predominated.

9. There is marked elevation of CIC values from Normal control to oral leukoplakia then to OSMF.

10. 4 homogenous and 3 non homogenous leukoplakia showed high CIC values which is above OD450nm -0.08 deviates greatly from the

mean value from the Group I leukoplakia which is 0.05988. This may be attributed to the fact that these lesions carry a high relative risk for malignant transformation.

11. In the same way OSMF subjects with clinical Grade IV showed high levels of CIC of OD 450 >0.2.

12. In the present study the mean CIC level is getting raised from normal control to oral leukoplakia subjects and then to Oral submucous fibrosis. There exists strong correlation among the three Groups with respect to the CIC value,which is denoted by ,

The p value between Group-I and Group-II is 0.0001 ($p<0.05$)

p value between Group I and GroupIII is 0.003 ($p<0.05$)

p value between Group-II and Group-III is 0.0001 ($p<0.05$).

To conclude the present study clearly shows the raise in CIC levels from,

NORMALCONTROLS→ORAL LEUKOPLAKIA→ORAL SUBMUCOUS FIBROSIS.

The stages of the malignancy can be determined by the levels of CIC level present in the serum. This can be correlated with histo pathological study and proper grading can be achieved.

It will be worthwhile a detailed study of OSMF correlating with their histopathology and CIC level in a larger sample size will bring forth in determining the stage of the cancer. This will enable to determine the therapy and the prognosis of the disease in a more précised manner.

When studying the larger sample size, the specific antigen for the tumour is identified and isolated from the serum, it will be helpful in treating the malignancy in a more specific manner to reduce the severity and also aids in curing of the disease.

These efforts may be of value for proactive intervention, especially in high risk groups with potentially malignant lesions and conditions, as the early detection decreases the morbidity and mortality of the diseases.

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RAGAS DENTAL COLLEGE & HOSPITAL

2/102, EAST COAST ROAD, Uthandi, Chennai – 600119

DEPARTMENT OF ORAL MEDICINE & RADIOLOGY

ESTIMATION OF CIRCULATING IMMUNE COMPLEXES IN PATIENTS WITH ORAL LEUKOPLAKIA AND ORAL SUBMUCOUS FIBROSIS-A CASE CONTROL STUDY.

Date:

S.No :

OP.No :

Study group : Group I / Group II / Group III

Name :

Age/Sex :

Address :

Phone number :

Occupation :

Monthly income :

Past medical /surgical/dental /history :

Chewing habits :

- Duration of chewing (<5yrs/ 5-10 yrs / 11-20 yrs / >21 yrs)

- Frequency of chewing per day (<5 times / 6-10times / >11times)

Smoking:

- Duration of smoking (<5yrs/ 5-10 yrs / 11-20 yrs / >21 yrs)
- Frequency of smoking per day (<5 times / 6-10times / >11times)

Alcohol consumption:

- Duration of alcohol consumption (<5yrs/5-10 yrs / 11-20 yrs / >21 yrs)
- Frequency of alcohol consumption per month (<5 times / 6-10times / >11times)

Leukoplakia :

Site :

Size :

Type :

Oral submucous fibrosis :

Grade :

Circulating Immune Complex level in OD 450 nm :

Date :
Place :

CONSENT LETTER

I _____ the undersigned hereby give my consent for the performance of diagnostic test on myself **"Estimation of Circulating Immune Complexes in patients with Oral Leukoplakia and Oral submucous fibrosis – A Case control study"** conducted by Dr. H. Maheswari under the able guidance of Dr.(Capt).S. Elangovan M.D.S., Professor, Department of Oral Medicine and Radiology, Ragas Dental College and Hospital, Chennai. I have been informed and explained the status of my disorder, evaluation procedure, risk involved and likelihood of success. I also understand and accept this as a part of study protocol, thereby voluntarily, unconditionally, freely give my consent without any fear of pressure in mentally sound and conscious state to participate in the study.

Witness : Patient Signature

ஒப்புதல் படிவம்

_____என்கின்ற நான், சென்னை, ராகாஸ் பல் மருத்துவக் கல்லூரி மற்றும் மருத்துவமனையின் வாய் மருத்துவம் மற்றும் ஊடுகதிர் துறையின் பேராசியர் மரு. கேப்டன். S.இளங்கோவன் அவர்களின் மேற்பார்வையில், முதுநிலை (M.D.S.) பட்டப்படிப்பு பயிலும் மரு. H. மஹேஸ்வரி அவர்கள் மேற்கொள்ளும் **"வாய் வெண்படலம் மற்றும் வாய் தசை இறுகிப் போகுதல் ஆகிய பிரிவுகளில் உள்ள நோயாளிகளின் இரத்தத்தில் உள்ள நோய் எதிர்ப்பு கூட்டு அணுக்களின் அளவை கண்டறிதல்"** என்கின்ற ஆராய்ச்சிக்கான பரிசோதனைகளுக்கு என்னை உட்படுத்துவதற்கு எனது மனமுவந்த பரிபூரண சம்மதத்தினை அளிக்கிறேன்.

மேலும் எனக்கு என்னுடைய நோயின் தன்மையைப்பற்றியும், அதனால் ஏற்படக்கூடிய விளைவுகளைப்பற்றியும் எடுத்துக் கூறப்பட்டுள்ளது எனவும், இந்த பரிசோதனைக்கு நான் எந்தவித அச்சமுமின்றி தன்னிச்சையாகவும், தெளிவான முழு மனதுடன் என்னுடைய பரிபூரண சம்மதத்தினை அளிக்கிறேன் என இதன் மூலம் தெரியபடுத்துகிறேன்.

சாட்சியாளர்கள்: இப்படிக்கு,